Applied and Environmental Microbiology, June 2008, p. 3672–3681 Vol. 74, No. 12
0099-2240/08/$08.00+0 doi:10.1128/AEM.02624-07

Longitudinal Study of Antimicrobial Resistance among *Escherichia coli* Isolates from Integrated Multisite Cohorts of Humans and Swine▽

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Received 20 November 2007/Accepted 10 April 2008

In a 3-year longitudinal study, we examined the relationship between the seasonal prevalence of antimicrobial-resistant (AR) *Escherichia coli* isolates from human wastewater and swine fecal samples and the following risk factors: the host species, the production type (swine), the vocation (human swine workers, non-swine workers, and slaughter plant workers), and the season, in a multisite, vertically integrated swine and human population representative of a closed agri-food system. Human and swine *E. coli* (n = 4,048 and 3,429, respectively) isolates from wastewater and fecal samples were tested for antimicrobial susceptibility, using the Sensititre broth microdilution system. There were significant ($P < 0.05$) differences among AR *E. coli* prevalence levels of (i) the host species, in which swine isolates were at higher risk for resistance to tetracycline, kanamycin, ceftiofur, gentamicin, streptomycin, chloramphenicol, sulfisoxazole, and ampicillin; (ii) the swine production group, in which purchased boars, nursery piglets, and breeding boars isolates had a higher risk of resistance to streptomycin and tetracycline; and iii) the vocation cohorts, in which swine worker cohort isolates exhibited lower sulfisoxazole and cefoxitin prevalence than the non-swine worker cohorts, while the slaughter plant worker cohort isolates exhibited elevated cefoxitin prevalence compared to that of non-swine workers. While a high variability was observed among seasonal samples over the 3-year period, no significant temporal trends were apparent. There were significant differences in the prevalence levels of multidrug-resistant isolates between host species, with swine at a higher risk of carrying multidrug-resistant strains than humans. Considering vocation, slaughter plant workers were at higher risk of exhibiting multidrug-resistant *E. coli* than non-swine workers.

The emergence, propagation, accumulation, and maintenance of strains of antimicrobial-resistant (AR) pathogenic bacteria have become a worldwide health concern in human and veterinary medicine (1, 9, 19). The intensive therapeutic uses and misuses of antimicrobial agents in humans and companion animals, as well as their therapeutic, prophylactic, and subtherapeutic uses for growth promotion in food animals, have substantially increased selective pressures on both pathogenic and commensal bacteria, thus favoring the propagation, accumulation, and maintenance of AR bacteria (8).

Many authors have attempted to link antimicrobial use in food animal agriculture with an increased risk of AR bacteria in humans (15, 18, 20). These authors have speculated that AR bacteria in animals could transfer to human populations through direct contact (e.g., occupational exposure) and through indirect contact with animals (e.g., consumption of contaminated food products of animal origin). However, those studies have been based largely on historical data and cross-sectional studies lacking a temporal component to establish cause-effect relationships.

In several antimicrobial resistance studies, assessments of human exposure to AR bacteria from animals and food products of animal origin, in relationship to resistance levels in human populations, have lacked the control of several essential factors including (i) open study populations, with limited or no control over the in- and out-migration of subjects (humans or animals) in the study areas; (ii) human travel and trade in animals and food products, which serve as a source for AR bacteria that can be introduced into susceptible populations; and (iii) lack of temporal components, as in cohort studies that require follow-up with individual or groups of animal and human subjects over a lengthy period of time (2, 4, 11, 12, 15, 16, 17).

The primary objective of this 3-year longitudinal study conducted at multiple sites and with a vertically integrated swine and human agri-food system, was to examine the relationship between the prevalence of AR commensal *Escherichia coli* isolates from human wastewater and swine fecal samples from group-level cohorts of humans and swine and the following potential risk factors: (i) host species (swine versus human), (ii) swine production type (e.g., breeding/gestation, farrowing, nursery, grower, or finisher pigs); (iii) human vocation (swine workers, non-swine workers, and slaughter plant workers); (iv) human consumers versus nonconsumers (and non-swine workers); and (v) season.

MATERIALS AND METHODS

Study design. The agri-food system that we studied represented multisite housing units consisting of vertically integrated populations of human workers (and potential pork consumers) and swine. Humans were housed in 19 purposively chosen and geographical locations (units) across the state of Texas. Twelve

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▽ Published ahead of print on 18 April 2008.

of these units had colocated swine operations. One location was colocated with a swine slaughter facility.

Human population. The human population consisted of a total of approximately 39,000 male individuals distributed over the 19 unit locations. The age of the male population within the study system ranged from 18 to 50+ years old, with the majority ranging between 30 and 49 years (3), and the average length of stay for each individual across all locations was 4.5 years. Based on occupational exposure (i.e., vocation) to swine and other factors, these populations were classified as either swine workers, non-swine workers, swine slaughter plant workers, or non-swine workers/nonconsumers. Swine workers, non-swine workers, and swine slaughter plant workers potentially had the opportunity to consume pork produced within the integrated system; therefore, only the non-swine workers/nonconsumers did not have the opportunity to consume pork produced within the system. There were 12 units where both swine workers and non-swine workers resided, 6 units with only non-swine workers, and only 1 unit with a swine slaughter plant facility. Greater than 90% of the human population consisted of individuals with no potential for vocational exposure to swine. There was limited movement of new residents into the system and limited movement out of the system (the average duration of stay was 4.5 years or about 1.85% turnover per month). There was also a non-swine worker/nonconsumer cohort that resided outside the agri-food system (at some of the unit locations); this cohort was sampled in order to more properly represent the general human population as a "negative control" group.

Swine population. The swine population consisted of approximately 26,000 to 28,000 pigs in any given month, located across the 12 unit locations. The 12 swine operations were composed of five farrow-to-finisher swine facilities and seven grower-to-finisher facilities. There were occasional (i.e., roughly every 6 months) movements of pigs into the system (purchased purebred and mixed breed boars). These swine were received into a single quarantined swine operation, where pigs were held for 4 weeks prior to moving to the farrow-finisher operations. In addition, there were some 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 slaughtered swine flowed vertically through farrow-to-finisher units (farrowing barns to the hot nursery, then on to the cold nursery, the grower, and last to the finisher barns) or else from the cold nursery to other stand-alone grower-finisher units and were then sent to slaughter, where pork products were processed and fed back to the human population housed within the system. When pigs arrived at the slaughter plant, they were lairaged in holding pens overnight before they were slaughtered the next day. For the purpose of data analysis, the swine population was categorized into seven production groups, as follows: (i) farrow-crated pigs (which included farrowing sows and their piglets), (ii) nursery piglets (which included both hot- and cold-nursery piglets), (iii) breeding/gestation females (which included breeding gilts, pregnant sows, and gilts), (iv) breeding boars, (v) quarantined boars (purchased boars held at the quarantine facility), (vi) grower-to-finisher pigs, and (vii) slaughtered pigs (pigs at the holding pens).

Sampling scheme: human population. Multiple composite wastewater grab samples (approximately 50 ml each) were collected from all 19 human unit locations. These wastewater samples were sewage samples taken from sewer holes known to drain lavatory facilities representative of the four different cohorts of humans described above. The sewage wastewater systems (one at each unit location) were closed; that is, they were not susceptible to a rain-water-dilution effect due to surface water runoff during storms. The samples were collected monthly over a period of 36 months (February 2004 to January 2007) by trained personnel at each unit location. The mean temperature across the study units over the 3 years was 19.7°C, ranging between 5.5 and 29.8°C. Typically, at each unit with a swine operation, three swine-worker wastewater samples (potential pork consumers), three non-swine worker (including both potential pork consumers as well as known nonconsumers) wastewater samples, and one mixed influent (draining from all the groups) sample were collected. At those units without a swine operation, four wastewater samples: three non-swine worker (both potential pork consumers and nonconsumers) samples draining from sewer holes and one mixed influent sample were collected. At the single unit with a slaughter plant, seven wastewater samples were collected, representing three non-worker sewer holes (potential pork consumers), three slaughter plant worker sewer holes (potential pork consumers), and one mixed influent. The specific sampling locations were chosen to differentiate among the occupational and consumer group-level cohorts. Typically, the number of wastewater samples collected per month was 116. The numbers of wastewater samples collected over the 3-year period were as follows: 2,837 samples from the units with swine operations, 802 samples from the units without swine operations, and 182 samples from the unit with a slaughter plant. Sample pickup and shipping from each unit were performed by a privately licensed and contracted laboratory (samples were first refrigerated immediately following collection and then transported on ice overnight to the USDA-ARS-Southern Plains Agricultural Research Center [USDA-ARS-SPARC], College Station, TX) for further analysis. This contracted laboratory also collected and processed wastewater samples for the facilities' environmental regulatory purposes.

Swine population. Composite fresh fecal floor samples (approximately 50 g each) and barn wash/prelagoon 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 to December 2006). Samples were kept at 4°C overnight until they were shipped to the USDA-ARS-SPARC laboratory. Swine pens at every unit containing each of the production stages of swine were sampled at least once every 3 months. Each composite fecal sample (50 g) was composed of equal proportions of fecal pats randomly sampled from multiple pens. Barn wash/prelagoon samples were obtained from collection points that drained the pens. Furthermore, composite fecal samples from the slaughter plant holding pens, the kill floor effluent, and the pork trim samples from the slaughter plant unit also were collected monthly. Approximately one-third of the total pork consumed by the human population was from imported pork trim that was fully processed into breakfast sausage (personal communication with the 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/prelagoon influent samples was 35. The number of monthly swine samples was somewhat variable over the study period due to changes in the number of pigs at different production stages in the operations over the study period.

Phenotypic analysis of antimicrobial-resistant *E. coli*: microbiological isolation of *E. coli* isolates. When samples arrived at the laboratory, 5 aliquots of each of the human and swine samples was frozen at −72°C; three with glycerol, at a ratio of 3:1 (sample:glycerol), and two without glycerol, for later analysis. At the time of microbiological analysis, frozen human wastewater samples (with glycerol) were thawed completely and 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 (Becton Dickinson and Company, Sparks, MD) for enrichment. This mixture was then incubated for 18 h at 37°C, streaked onto a selective medium of CHROMagar *E. coli* (DRG International, Mountainside, NJ) and incubated further at 37°C for 18 to 24 h. No enrichment step was used when thawed swine fecal samples were cultured (i.e., these were streaked directly onto CHROMagar). For pork trim, approximately 5 g was dissected from the pork trim sample, mixed for 1 min in a stomacher with peptone water (10 ml), and then streaked with a sterile loop onto CHROMagar as described above.

A single typical *E. coli* colony (blue color with a smooth surface) was randomly selected from the CHROMagar plate, streaked onto a blood agar plate, and then incubated (18 h, 37°C). *E. coli* isolates on the blood agar were used for antimicrobial susceptibility testing (see below), and the remaining *E. coli* isolates were transferred onto glycerol-coated beads (Key Scientific, Round Rock, TX) and stored at −72°C for future retrospective analyses. Based on a pilot study for this research project (13), CHROMagar *E. coli*-selective medium was noted to be highly specific and yielded >98% *E. coli* colonies. Furthermore, a biochemical test strip (API 20E; BioMérieux, Inc.) was used regularly as a quality control to confirm the isolated bacteria were *E. coli*.

Determination of antimicrobial susceptibility for *E. coli* isolates. An *E. coli* isolate was picked from the blood agar culture as described above, and the MIC was determined for different antimicrobial agents by broth microdilution following CLSI (formerly NCCLS) standards (10). Antimicrobial susceptibility testing was performed with a Sensititre automated system, according to the manufacturer's instructions (Trek Diagnostic Systems, Cleveland, OH), using custom panels designed by the National Antimicrobial Resistance Monitoring System (NARMS) (7). The MICs for the *E. coli* isolates tested were determined to be either resistant or susceptible based on CLSI breakpoints or else based on NARMS committee consensus, where no CLSI breakpoint existed (10). Intermediate MIC results were reclassified as susceptible. To assess the variability in the number of different resistant phenotypes in a sample, five *E. coli* colonies were randomly selected from human and swine samples during 1 month only (February 2005). The mean number of different phenotypes per plate 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 used in the NARMS (7) panels and their breakpoints are shown in Table 1. The MIC ranges included in Table 1 represent the antimicrobial agent dilutions for the NARMS gram-negative bacteria panel; hence, the dilutions and breakpoints used in this study. Quality control organisms from the American Type Culture Collection

TABLE 1. Tabulated ranges of dilutions and breakpoints[a]

Antimicrobial agent	Range(s)	Breakpoint(s)
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[b]	16–512	≥512
Tetracycline	4–32	≥16
Trimethoprim-sulfamethoxazole	0.12–4	≥4

[a] Values show tabulated ranges and breakpoints for the 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.

[b] 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.

(ATCC; Manassas, VA), *E. coli* strains 25922 and 35218, *Enterococcus faecalis* 29212, and *Pseudomonas aeruginosa* 27853, were evaluated with approximately every 200 NARMS custom panels (or, when a new batch, as determined by serial number, of plates was begun); this quality control check was not necessarily made on a daily basis. Based on our pilot study (13), the consistency of the antimicrobial susceptibility quality control testing was very high.

Sample phenotypic analysis scheme. Samples were analyzed on a quarterly basis over the 3-year study period. All human and swine samples collected during the first 12 months of the study were phenotypically analyzed. These data were later collapsed into four seasons (or yearly quarters) based on (i) winter (February to April), (ii) spring (May to July), (iii) summer (August to October), and (iv) autumn (November to January). Thereafter, only quarterly phenotypic analysis was performed with the other 24 months of sampling. Quarterly sampling and analysis were conducted because the highest variability observed was between seasons rather than between months within a season, based on the first 12 months of data analysis.

Statistical analysis: descriptive statistics. The 15 antimicrobial-resistant *E. coli* outcomes (binary), as well as the multidrug 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, slaughter plant workers, and non-swine workers), human consumer versus nonconsumer, and season and year. Initially, the proportion of bacterial isolates resistant to each of the antimicrobial agents was compared across the levels of each risk factor, using either a two-sided 2-by-2 Fisher's exact test or 2-by-*n* likelihood ratio chi-square test, as appropriate, with STATA software version 9.2 (Stata Corp., College Station, TX). Multidrug resistance was assessed for each risk factor as the sum of resistance (out of 15 agents, the upper [6+] categories were collapsed) across all isolates, using an $m \times n$ likelihood ratio chi-square test.

Multivariable analysis of risk factors for *E. coli* resistance to individual antimicrobial agents. The association between each of the individual AR *E. coli* phenotypes and the risk factors in the study was assessed using a generalized linear model, with binomial error distribution and logit link function and adjusted for dependency within each unit location, using a generalized estimated equation (GEE) in STATA version 9.2 software.

Multivariable analysis of risk factors for *E. coli* resistance to multiple antimicrobial agents. The ordinal response (multidrug resistance from 0 to 6+) of *E. coli* phenotypes in relation to the risk factors was assessed by using a generalized linear model model, with a multinomial distribution and a cumulative logit link function and adjusted for dependency using GEE within each unit location in SAS version 9.1 software (PROC GENMOD; SAS Institute, Inc., Cary, NC). Multidrug resistance outcomes >6 were collapsed with response number 6 (called 6+) because of the rare resistance phenotypes with very sparsely populated cells for some the outcomes.

TABLE 2. Comparison of phenotypic resistance of commensal enteric *E. coli* isolates sampled across human vocation groups, swine production groups, and seasons[a]

Host species[b]	Antimicrobial agent	OR (*P* value)[c]	Likelihood ratio chi-square test *P* value[d]
Swine	Amikacin	2.59 (0.34)	0.154
Human			0.923
Swine	Amoxicillin-clavulanic acid	0.72 (0.047)	<0.001
Human			0.146
Swine	Ampicillin	1.22 (0.004)	<0.001
Human			<0.001
Swine	Cefoxitin	0.62 (0.006)	<0.001
Human			<0.001
Swine	Ceftiofur	5.56 (<0.001)	<0.001
Human			0.035
Swine	Ceftriaxone	1.08 (0.99)	0.761
Human			0.923
Swine	Chloramphenicol	2.02 (<0.001)	<0.001
Human			0.015
Swine	Ciprofloxacin	0.19 (0.004)	0.976
Human			<0.001
Swine	Gentamicin	3.65 (<0.001)	<0.001
Human			0.005
Swine	Kanamycin	9.41 (<0.001)	<0.001
Human			<0.001
Swine	Nalidixic acid	0.27 (<0.001)	0.555
Human			<0.001
Swine	Streptomycin	3.52 (<0.001)	<0.001
Human			<0.001
Swine	Sulfisoxazole	2.40 (<0.001)	<0.001
Human			<0.001
Swine	Tetracycline	18.78 (<0.001)	<0.001
Human			<0.001
Swine	Trimethoprim-sulfamethoxazole	0.15 (<0.001)	0.074
Human			0.006

[a] The odds ratios (ORs) and *P* values, as well as the likelihood ratio *P* values, for the proportion of *E. coli* isolates are presented and contrasted by host species. Isolates are compared across all human vocation cohorts, swine production groups, and seasons.

[b] Host species: swine, $n = 3{,}429$; human, $n = 4{,}048$.

[c] Odds ratio values present a comparison of the odds of prevalence for each phenotype of antimicrobial resistance in swine versus that of human *E. coli* isolates. *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 version 9.2 software, College Station, TX).

[d] *P* values are based on a likelihood ratio (χ^2 test) of the differences in risk across all units that were sampled. These data are presented and analyzed by host species.

Multivariate analysis (accounting for dependence among multiple binary outcomes). 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 software macro to adjust for dependence among the isolate resistance phenotypes (i.e., pharmacologic and biological or genetic dependence) and dependence within a unit location. Pharmacologic dependence can arise since multiple AR outcomes reflect similar classes of pharmaceuticals on the NARMS panel (e.g., cephalosporins such as cefoxitin, ceftiofur, and ceftriaxone). Genetic (biological) dependence might arise when genes that code for multiple AR outcomes are colocated on the same bacterial genetic element (e.g., on a plasmid). This SAS macro was adapted from Shelton et al. (14) and modified to perform the analysis of our data. The antimicrobial agent odds ratios for AR *E. coli* isolates (unadjusted versus adjusted for dependence among resistance phenotypes) were examined for (i) host species (swine versus human [referent]) and (ii) human swine workers, slaughter plant workers, non-swine workers/nonconsumers, isolation boars, breeding boars, farrowing sows and piglets, breeding/gestation females, grower-finisher pigs, and nursery pigs compared to that of the non-swine worker cohort (the referent).

TABLE 3. Phenotypic resistance of commensal *E. coli* isolates sampled across human vocation groups[a]

Antimicrobial	No. of *E. coli* isolates sampled by human vocation group (% of total)					*P* value[b]
	Non-swine workers/ nonconsumers (n = 528)	Swine workers (n = 1,131)	Non-swine workers (n = 1,675)	Slaughter plant workers (n = 307)	Influent (mixed isolates) (n = 252)	
Amikacin	0 (0.0)	0 (0.0)	2 (0.1)	0 (0.0)	0 (0.0)	0.497
Amoxicillin-clavulanic acid	29 (5.5)	22 (2.0)	41 (2.5)	12 (3.9)	5 (2.0)	0.002
Ampicillin	135 (25.6)	152 (13.4)	245 (14.6)	49 (16.0)	42 (16.7)	<0.001
Cefoxitin	34 (6.4)	17 (1.5)	45 (2.7)	16 (5.2)	6 (2.4)	<0.001
Ceftiofur	0 (0.0)	4 (0.4)	7 (0.4)	3 (1.0)	0 (0.0)	0.497
Ceftriaxone	0 (0.0)	0 (0.0)	2 (0.1)	0 (0.0)	0 (0.0)	0.092
Chloramphenicol	9 (1.7)	24 (2.1)	27 (1.6)	2 (0.7)	2 (0.8)	0.258
Ciprofloxacin	10 (1.9)	8 (0.7)	13 (0.8)	0 (0.0)	0 (0.0)	0.005
Gentamicin	0 (0.0)	6 (0.5)	9 (0.5)	5 (1.6)	0 (0.0)	0.012
Kanamycin	16 (3.0)	12 (1.1)	20 (1.2)	11 (3.6)	0 (0.0)	<0.001
Nalidixic acid	24 (4.6)	54 (4.8)	94 (5.6)	1 (0.3)	14 (5.6)	<0.001
Streptomycin	55 (10.4)	100 (8.8)	141 (8.4)	38 (12.4)	25 (9.9)	0.209
Sulfisoxazole	67 (12.7)	114 (10.1)	224 (13.4)	42 (13.7)	31 (12.3)	0.099
Tetracycline	98 (18.6)	231 (20.4)	300 (18.0)	102 (33.2)	57 (22.6)	<0.001
Trimethoprim-sulfamethoxazole	45 (8.5)	74 (6.5)	125 (7.5)	33 (10.8)	13 (5.2)	0.069

[a] Phenotypic resistance of commensal *E. coli* isolates from human wastewater samples (n = 3,893 isolates, with vocation cohorts identified). Frequencies and proportions are contrasted with human vocation cohorts across all unit locations and seasons.

[b] *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.

RESULTS

Descriptive statistics. There were 7,477 (4,048 human and 3,429 swine) commensal *E. coli* isolates collected from the wastewater and fecal matter samples over the 3-year study period. Due to occasional scheduling conflicts, not all units were sampled for wastewater collection every month. The 15 antimicrobial resistance outcomes (binary, susceptible or resistant) for *E. coli* isolates were cross-tabulated by host species (Table 2). The individual antimicrobial resistance *E. coli* phenotypes cross-tabulated by human vocation cohort (i.e., the non-swine workers/nonconsumers, swine workers, non-swine workers, influent mixture, and slaughter plant workers), are shown in Table 3. The individual phenotypes cross-tabulated by swine production group (i.e., slaughter plant holding pens [slaughtered pigs], breeding boars, isolation/quarantined boars, breeding sows, farrowing sows and piglets, growers and finishers, and nursery piglets) are shown in Table 4. There were 12 *E. coli* bacterial strains isolated from the 160 pork trim samples (7.5%), 11 of which were resistant to at least one antimicrobial agent. Among those isolates, the total frequency of multidrug-resistant phenotypes was as follows: pansusceptible, n = 1; single-agent-resistant isolate, n = 4; and resistant to three antimicrobial agents, n = 7; with the most common phenotypes of resistance to tetracycline and ampicillin-strep-

TABLE 4. Phenotypic resistance of commensal *E. coli* isolates sampled from swine production groups[a]

Antimicrobial agent	No. of *E. coli* isolates sampled by swine production group (% of total)							*P* value[b]
	Slaughter holding pigs (n = 72)	Breeding boars (n = 195)	Quarantined boars (n = 331)	Breeding/gestation females (n = 131)	Farrowing sows and piglets (n = 755)	Grower and finisher pigs (n = 1,576)	Nursery piglets (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)	<0.001
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)	<0.001
Ceftiofur	1 (1.4)	1 (0.5)	3 (0.9)	0 (0.0)	24 (3.2)	28 (1.8)	26 (7.1)	<0.001
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)	<0.001
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)	<0.001
Kanamycin	4 (5.6)	23 (11.8)	113 (34.1)	10 (7.6)	67 (8.9)	102 (6.5)	82 (22.3)	<0.001
Nalidixic acid	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	3 (0.2)	0 (0.0)	<0.001
Streptomycin	11 (15.3)	55 (28.2)	115 (34.7)	21 (16.0)	232 (30.7)	308 (19.5)	175 (47.5)	<0.001
Sulfisoxazole	12 (16.7)	30 (15.4)	179 (54.1)	18 (13.7)	179 (23.7)	325 (20.6)	165 (44.8)	<0.001
Tetracycline	46 (63.9)	176 (90.3)	325 (98.2)	107 (81.7)	622 (82.4)	1,310 (83.1)	335 (91.0)	<0.001
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] Phenotypic resistance of commensal *E. coli* isolates among swine fecal sample (n = 3,429 isolates with production groups identified). Frequencies are contrasted by swine production groups across all unit locations and seasons.

[b] *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.

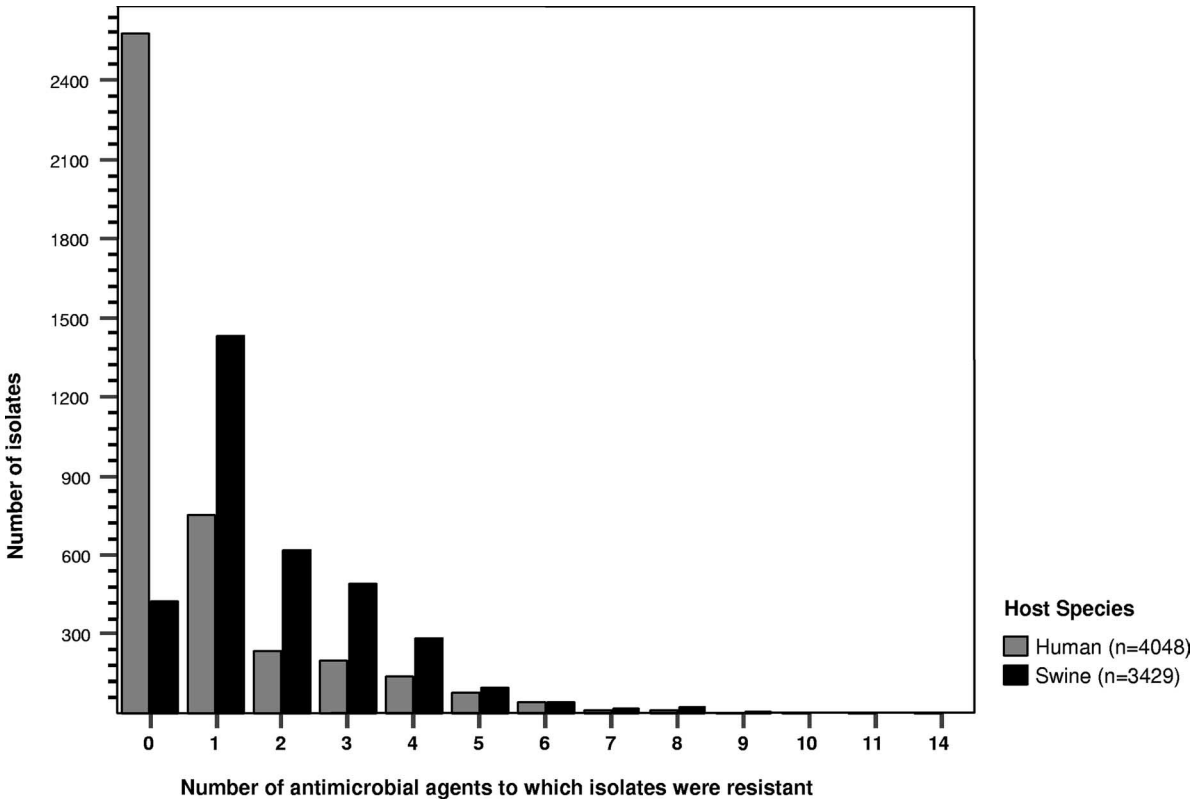

FIG. 1. Frequency bar chart illustrating the distribution of phenotypic resistance to up to 15 antimicrobials among *E. coli* isolates from human and swine samples.

tomycin-tetracycline. Results were also cross-tabulated by consecutive seasons (winter, spring, summer, and autumn) throughout the 3-year period (data not shown). In general, high variability was observed among seasonal samples over the 3-year period for both human and swine isolates across all units; however, no long-term trends (e.g., changes in prevalence over 3 years) were apparent.

Approximately 63% of the human *E. coli* isolates were pansusceptible to the 15 antimicrobial agents with the NARMS panel, 18.6% were single-agent-resistant isolates, and 17.8% were resistant to two or more antimicrobial agents. In contrast, only 12.4% of the swine *E. coli* isolates were pansusceptible, whereas 41.8% were single-agent-resistant isolates, and 45.7% were resistant to two or more antimicrobial agents. The distribution of multidrug-resistant *E. coli* phenotypes is not collapsed into the 6+ categories in Fig. 1 in order to better show the maximum multidrug resistance phenotypes for *E. coli* isolates arising from human and swine samples. The multidrug resistance phenotypes for *E. coli* isolates were cross-tabulated by season (winter, spring, summer, and autumn) across all 3 years and host species (Table 5). High variability was observed among seasonal samples for the multidrug resistance phenotypes over the 3-year period for both human and swine samples.

Multidrug resistance totals, cross-tabulated by human vocation cohorts and swine production groups, are shown in Table 6. The multidrug-resistant *E. coli* isolates from human samples differed significantly (i) among human vocation cohorts ($\chi^2 = 69.9$; $P < 0.001$) and (ii) among swine production groups ($\chi^2 = 495.2$; $P < 0.001$) across all levels of multidrug resistance.

Multivariable analysis of risk factors for *E. coli* resistance to individual antimicrobial agents. Comparison of *E. coli* resistance to individual antimicrobial agents between host species. The odds ratios and associated *P* values for *E. coli* resistance, adjusted for the dependence of host species isolate response within each unit location, comparing swine isolates to that of humans, are shown in Table 2.

Comparison of *E. coli* resistance to individual antimicrobial agents by swine production group. The odds ratios (ORs) for *E. coli* resistance adjusted for the dependence of the swine production group isolate response within each unit location were significantly increased ($P < 0.05$) for quarantined boar, nursery piglet, and breeding boar *E. coli* isolates compared to those of the swine referent group (slaughtered pigs) for tetracycline (OR = 12.25 [95% confidence interval {CI }, 2.41 to 62.15]; OR = 3.50 [95% CI, 1.08 to 11.27]; and OR = 3.20 [95% CI, 0.97 to 10.55], respectively) and streptomycin (OR = 2.90 [95% CI, 1.42 to 5.95]; OR = 4.96 [95% CI, 2.46 to 9.99]; and OR = 2.14 [95% CI, 1.02 to 4.50], respectively). Furthermore, the ORs of *E. coli* resistance adjusted for the dependence of swine production group isolate response within each unit location were significantly increased ($P < 0.05$) for isolation boar and nursery piglet *E. coli* isolates compared to those of the swine referent group (slaughtered pigs) for sulfisoxazole (OR = 5.83 [95% CI, 3.01 to 11.34] and 4.06 [95% CI, 2.10 to 7.85], respectively). Table 4 provides the proportion of resis-

tant *E. coli* isolates by swine production group for each antimicrobial agent.

Comparison of *E. coli* resistance to individual antimicrobial agents by human vocation cohort. The ORs for *E. coli* resistance adjusted for the dependence of human vocation cohort isolate response within each unit location were significantly increased ($P < 0.05$) among non-swine worker/nonconsumer isolates for amoxicillin-clavulanic acid (OR = 2.28 [95% CI, 1.38 to 3.77]), ampicillin (OR = 1.83 [95% CI, 1.40 to 2.39]), and cefoxitin (OR = 2.25 [95% CI, 1.35 to 3.75]) compared to those of non-swine workers. Furthermore, the ORs of *E. coli* resistance adjusted for the dependence of human vocation cohort *E. coli* isolate response within each unit location were significantly increased ($P < 0.05$) among slaughter plant worker isolates for cefoxitin (OR = 2.38 [95% CI, 1.10 to 5.17]) compared to those of non-swine workers. Moreover, the ORs for *E. coli* resistance among swine workers adjusted for the dependence of human vocation cohort isolate response within each unit location were significantly lower ($P < 0.05$) for sulfisoxazole (OR = 0.75 [95% CI, 0.59 to 0.95]) and cefoxitin (OR = 0.47 [95% CI, 0.25 to 0.88]) compared to those of non-swine workers. The ORs for *E. coli* resistance, adjusted for the dependence of human vocation cohort isolate response within each unit location among pork consumer (i.e., swine workers, non-swine workers, and slaughter plant workers) *E. coli* isolates were significantly lower ($P < 0.05$) for amoxicillin-clavulanic acid (OR = 0.42 [95% CI, 0.27 to 0.66]), ampicillin (OR = 0.53 [95% CI, 0.41 to 0.67]), cefoxitin (OR = 0.37 []95% CI, 0.23 to 0.59]), ciprofloxacin (OR = 0.39 [95% CI, 0.16 to 0.92]), and kanamycin (OR = 0.43 [95% CI, 0.22 to 0.86]) compared to those of the non-swine worker/nonconsumer group cohorts.

Comparison of *E. coli* resistance to individual antimicrobial agents by season. There were small changes observed among the seasonal isolates (i.e., overall seasons, collapsed by season and year, and collapsed by season-by-year interaction) for both human and swine isolates over the 3-year study period (data not shown). However, no repeatable seasonal trend was detected, nor was there a long-term trend showing change of prevalence over the 3-year period. This conclusion was based on the very high variability and instability in the GEE model parameter estimates for each of the 15 antimicrobial resistance outcomes for *E. coli* in relation to the seasons.

Multivariable analysis of risk factors for *E. coli* resistance to multiple antimicrobial agents. Comparison of *E. coli* resistance to multiple antimicrobial agents by host species. The multinomial ORs for the total multidrug resistance phenotype adjusted for the dependence of host species isolate response within each unit location were significantly increased ($P < 0.05$) among swine isolates (OR = 6.47 [95% CI, 4.29 to 9.76]) compared to those of human isolates across all levels of multidrug resistance.

Comparison of *E. coli* resistance to multiple antimicrobial agents by swine production group. The multinomial ORs for the total multidrug resistance phenotype adjusted for the dependence of swine production group isolate response within each unit location did not differ significantly ($P > 0.05$) by swine production group.

Comparison of *E. coli* resistance to multiple antimicrobial agents by human vocation cohort. The multinomial ORs for

TABLE 5. Multidrug resistance phenotypes of commensal *E. coli* isolates sampled by year and season[a]

Multidrug resistance	Host species (n)[c]	No. of multidrug-resistant *E. coli* isolates sampled by year and season (% of total)[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	Human (2,575)	286 (62.86)	296 (68.68)	281 (63.15)	272 (61.26)	280 (49.82)	186 (66.91)	178 (66.17)	143 (50.71)	186 (80.17)	214 (80.15)	97 (64.67)	156 (67.24)
	Swine (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	Human (751)	96 (21.10)	73 (16.94)	82 (18.43)	87 (19.59)	119 (21.17)	46 (16.55)	36 (13.38)	83 (29.43)	23 (9.91)	34 (12.73)	35 (23.33)	37 (15.95)
	Swine (1,433)	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	Human (234)	23 (5.05)	22 (5.10)	29 (6.52)	23 (5.18)	66 (11.74)	15 (5.40)	14 (5.20)	18 (6.38)	4 (1.72)	6 (2.25)	7 (4.67)	7 (3.02)
	Swine (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	Human (199)	18 (3.96)	13 (3.02)	30 (6.74)	24 (5.41)	30 (5.34)	20 (7.19)	21 (7.81)	18 (6.38)	10 (4.31)	6 (2.25)	0 (0.00)	8 (3.45)
	Swine (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	Human (138)	19 (4.18)	10 (2.32)	13 (2.92)	14 (3.15)	37 (6.58)	6 (2.16)	4 (1.49)	7 (2.48)	4 (1.72)	4 (1.50)	4 (2.67)	16 (6.90)
	Swine (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	Human (81)	9 (1.98)	6 (1.39)	7 (1.57)	12 (2.70)	17 (3.02)	4 (1.44)	6 (2.23)	9 (3.19)	3 (1.29)	1 (0.37)	3 (2.00)	4 (1.72)
	Swine (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)
6+[d]	Human (70)	4 (0.88)	11 (2.55)	3 (0.67)	12 (2.70)	13 (2.31)	1 (0.36)	10 (3.72)	4 (1.42)	2 (0.86)	2 (0.75)	4 (2.67)	4 (1.72)
	Swine (83)	21 (3.99)	7 (1.76)	17 (4.21)	12 (3.04)	6 (1.15)	3 (1.96)	5 (2.14)	1 (0.74)	2 (1.38)	3 (1.57)	3 (2.00)	3 (1.69)

[a] Multidrug resistance phenotypes of commensal *E. coli* isolates (n = 7,477 overall sample isolates) from human wastewater and swine fecal samples. The frequencies and percentages of multidrug resistant *E. coli* isolates are contrasted with host species and seasons. Isolate comparisons are across all unit locations, human vocation cohorts, and swine production groups.

[b] Differences in multidrug-resistant *E. coli* human isolates and seasons were significant ($\chi^2 = 249.8$, $P < 0.001$) using m-by-n likelihood-ratio chi-square test. Likewise, the differences in multidrug-resistant *E. coli* swine isolates and seasons was significant ($\chi^2 = 206.3$, $P < 0.001$) using m-by-n likelihood-ratio chi-square test. Both P values are not adjusted for the dependence of responses within unit locations.

[c] Number of *E. coli* isolates for human and swine for each multidrug resistance category.

[d] Multidrug resistance to greater than six antimicrobial agents (6+) was collapsed into a single upper category.

TABLE 6. Multidrug resistance phenotypes of commensal *E. coli* isolates sampled across human vocation and swine production groups[a]

Multidrug resistance response	No. of multidrug-resistant *E. coli* isolate (% of total) samples by[b]:										
	Human vocation group				Swine production group						
	Non-swine workers/ nonconsumers (n = 528)	Swine workers (n = 1,131)	Non-swine workers (n = 1,675)	Influent (mixed isolates) (n = 252)	Slaughter holding pigs (n = 307)	Breeding boars (n = 195)	Quarantined boars (n = 331)	Breeding/ gestation females (n = 131)	Farrowing sows and piglets (n = 755)	Grower and finisher pigs (n = 1,576)	Nursery piglets (n = 368)
0	309 (58.52)	747 (66.05)	1,100 (65.67)	158 (62.70)	24 (33.33)	15 (7.69)	4 (1.21)	20 (15.27)	110 (14.57)	233 (14.78)	18 (4.89)
1	99 (18.75)	203 (17.95)	295 (17.61)	47 (18.65)	29 (40.28)	85 (43.59)	67 (20.24)	69 (52.67)	283 (37.48)	780 (49.49)	120 (32.61)
2	32 (6.06)	67 (5.92)	82 (4.90)	21 (8.33)	10 (13.89)	46 (23.59)	73 (22.05)	24 (18.32)	127 (16.82)	284 (18.02)	55 (14.95)
3	45 (8.52)	52 (4.60)	74 (4.42)	10 (3.97)	4 (5.56)	34 (17.44)	70 (21.15)	11 (8.40)	141 (18.68)	165 (10.47)	68 (18.48)
4	18 (3.41)	23 (2.03)	67 (4.00)	8 (3.17)	3 (4.17)	10 (5.13)	75 (22.66)	7 (5.34)	60 (7.95)	74 (4.70)	53 (14.40)
5	12.00 (2.27)	18 (1.59)	28 (1.67)	5 (1.98)	1 (1.39)	5 (2.56)	30 (9.06)	0 (0.00)	13 (1.72)	27 (1.71)	18 (4.89)
6+[c]	13 (2.46)	21 (1.86)	29 (1.73)	3 (1.19)	1 (1.39)	0 (0.00)	12 (3.63)	0 (0.00)	21 (2.78)	13 (0.82)	36 (9.78)

[a] Multidrug resistance phenotypes of commensal *E. coli* isolates (n = 7,477 overall sample isolates) from human and swine fecal isolates (human and swine cohorts). Frequencies and percentages of multidrug-resistant *E. coli* isolates are presented and contrasted with those of human vocation cohorts and swine production cohorts. Isolates are compared across all unit locations and seasons.

[b] Multidrug-resistant *E. coli* isolates from human samples differed significantly (χ^2 = 69.9, $P < 0.001$) among human vocation cohorts across all levels of multidrug resistance, using m-by-n likelihood-ratio chi-square test. Likewise, the multidrug-resistant *E. coli* isolates from swine samples differed significantly (χ^2 = 495.2, $P < 0.001$) among swine production groups across all levels of multidrug resistance. Both P values are not adjusted for the dependence of responses within unit locations.

[c] Multidrug resistance to greater than six antimicrobial agents (6+) was collapsed into a single upper category.

total multidrug resistance adjusted for the dependence of human vocation cohort isolate response within each unit location were significantly ($P < 0.05$) increased among slaughter plant worker isolates (OR = 1.70 [95% CI, 1.44 to 1.90]) compared to those of the referent (non-swine workers) isolates.

Comparison of *E. coli* resistance to multiple antimicrobial agents by season. There were small changes observed among the seasonal isolates (i.e., overall seasons, collapsed by season and year, and collapsed by season-by-year interaction) for both human and swine isolates over the 3-year study period (Table 7 shows the ORs of multidrug-resistant *E. coli* isolates by host species and season). However, once again, no repeatable seasonal trend was detected. Table 5 provides the proportion of multidrug-resistant (1 to 6+ categories) *E. coli* isolates by season of human and swine isolates for each antimicrobial agent.

Multivariate analysis of risk factors for *E. coli* resistance to multiple outcomes: by host species. The multivariate ORs adjusted for multiple binary outcomes (i.e., multivariate individual phenotypes per isolate) were variable (that is, both increased or decreased) relative to the unadjusted ORs among swine isolates compared to those among human isolates (data not shown).

Multivariate analysis by human cohorts and swine production groups. Seven antimicrobial agents' resistance outcomes 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' resistance outcomes included in the dependent term for the model. All of these antimicrobial agents' resistance outcomes were from rare resistance phenotypes with very sparsely populated cells for some categories. Therefore, only eight antimicrobial agents' resistance outcomes were included in the final analysis (Table 8). The adjusted ORs of multiple binary outcomes (i.e., multivariate phenotypes) were decreased and became nonsignificant ($P > 0.05$) relative to the unadjusted (for multivariate outcomes) ORs among slaughter plant worker isolates compared to non-swine workers isolates only for ampicillin (OR = 1.17 versus 1.04). The changes in adjusted ORs relative to the unadjusted ORs (increased or decreased) were variable among swine workers, slaughter plant worker, non-swine worker/nonconsumer, and swine production group isolates compared to that of non-swine workers (see the comparison in Table 8).

DISCUSSION

To our knowledge, the present study is the first that longitudinally assesses the risk for the elevated prevalence of AR bacteria in human populations in an integrated human and swine system as a result of direct occupational exposure to animals (i.e., swine).

TABLE 7. Multidrug resistance phenotypes of commensal *E. coli* isolates sampled by year and season over 3 years[a]

Host species (n)	Odds ratio of resistant *E. coli* isolates sampled by year and season (P value)[b]										
	Spring 2004	Summer 2004	Autumn 2004	Winter 2005	Spring 2005	Summer 2005	Autumn 2005	Winter 2006	Spring 2006	Summer 2006	Autumn 2006
Human (4,048)	1.05 (0.77)	1.01 (0.98)	0.97 (0.91)	1.86 (0.01)	0.41 (0.01)	0.80 (0.15)	0.85 (0.47)	0.42 (0.001)	1.17 (0.25)	1.49 (0.12)	0.92 (0.76)
Swine (3,429)	1.49 (0.001)	2.03 (0.12)	0.88 (0.62)	0.71 (0.04)	0.70 (0.02)	0.97 (0.75)	0.90 (0.42)	1.14 (0.66)	1.30 (0.02)	0.90 (0.62)	1.04 (0.93)

[a] Multidrug resistance phenotypes of commensal *E. coli* isolates from human wastewater and swine fecal samples. The odds ratios of multidrug-resistant *E. coli* isolates are presented and contrasted by host species and season. Isolates are compared across all unit locations, human vocation cohorts, and swine production groups. Odds ratios are presented comparing the odds of seasonal multidrug-resistant *E. coli* isolates to winter 2004 (the referent season). Multidrug resistance values greater than 6 antimicrobial agents (6+) were collapsed into a single upper-level category.

[b] Total n = 7,477 overall sample isolates. P values are adjusted for the dependence of seasonal isolate responses within each unit location by using the generalized estimating equation (GEE) statistic (PROC GENMOD; SAS Institute, Inc., Cary, NC). The overall chi-square statistic for the season was not significant for human isolates (χ^2 = 15.92, P = 0.14), as well as for swine isolates (χ^2 = 11.79, P = 0.38).

In our study, the higher levels of *E. coli* resistance in swine isolates than in human isolates are likely associated with either the past or current use of injectable antimicrobial agents (e.g., ceftiofur sodium) or the use of antimicrobial agents in feed (e.g., chlortetracycline) or water on a larger scale than that used in human medicine. On the other hand, human isolates had higher levels of resistance than swine isolates to five individual antimicrobial agents (ciprofloxacin, trimethoprim-sulfamethoxazole, nalidixic acid, cefoxitin, and amoxicillin-clavulanic acid), which might be explained by the use of these agents mainly in human medicine and rarely, if ever, in the swine industry (especially in our study population). We have ongoing analyses to evaluate the relationship between AR levels and the recent and concurrent use of antimicrobial agents in both host species. However, there are no data concerning longer-range historical use of the antimicrobial agents in these two populations. The historical use of the antimicrobial agents, in the relatively short time frame of the study, is only one component of the risk factors and is not the focus of the present paper. Short-term fluctuations in antimicrobial use may well be predictive of the short-term AR fluctuations in relatively newly introduced drugs but do not tend to explain macro trends in AR within swine, as evidenced by the small reductions in resistance seen with organic versus conventional swine operations in the United States. (6).

Swine fecal *E. coli* isolates exhibiting multidrug resistance were present at higher levels than human isolates. The higher levels of multidrug resistance in the swine population than in the human population might be attributed to several factors: (i) the prophylactic/subtherapeutic use of several antimicrobial agents in feed at the swine farms and (ii) the intensive farm management practices on swine farms that may facilitate the transmission, propagation, and maintenance of the AR bacterial populations in both the swine hosts and the farm environment.

The adjusted ORs of resistance were significantly increased among non-swine worker/nonconsumer isolates for amoxicillin-clavulanic acid, ampicillin, and cefoxitin compared to those of non-swine workers. The non-swine workers/nonconsumers represented a negative control group that resided outside of the agri-food study system. Therefore, these AR levels may better reflect the resistance levels in the general population compared to those in our within-system human populations. The non-swine worker/nonconsumer group was an open population in which there was (i) limited or no control over the in- and out-migration of humans; (ii) limited or no control over human travel and trade (animals and food products), which serve as a source for AR bacteria that can be introduced into this population; and (iii) limited or no control over food consumption from unknown sources. However, this cohort was located near the other two populations.

The adjusted ORs of resistance were significantly increased among slaughter plant worker isolates for cefoxitin compared to those of non-swine workers (the referent group). Furthermore, the adjusted ORs of multidrug resistance were significantly increased among slaughter plant worker isolates compared to those of the non-swine workers. This might be attributed to the overall high resistance prevalence in the slaughtered pigs and the greater likelihood of slaughter workers' exposure to slaughtered pigs' fecal matter, pigs' skin con-

TABLE 8. Multivariate resistance phenotypes of commensal *E. coli* isolates sampled across human vocation groups and swine production groups[a]

Antimicrobial agent	Adjusted for dependence among binary outcomes[b]	ORs of multivariate resistance *E. coli* phenotypes (95% CI)								
		Human vocation group			Swine production group					
		Swine worker isolates	Non-swine workers/ nonconsumers	Slaughter plant workers	Quarantined boars	Breeding boars	Farrowing sows and piglets	Breeding/gestation females	Grower and finisher pigs	Nursery piglets
Amoxicillin-clavulanic acid	No	0.8 (0.53–1.20)	2.34 (1.60–3.41)	1.69 (1.24–2.28)	0.79 (0.60–1.05)	0.2 (0.03–1.37)	1.63 (0.77–3.48)	0.32 (0.06–1.80)	0.32 (0.21–0.50)	2.91 (0.77–11.00)
	Yes	0.73 (0.47–1.13)	2.13 (1.57–2.88)	1.41 (1.08–1.83)	0.98 (0.77–1.24)	0.13 (0.01–2.13)	1.3 (0.66–2.60)	0.23 (0.02–2.35)	0.32 (0.21–0.48)	2.4 (0.62–9.60)
Ampicillin	No	0.86 (0.64–1.14)	1.74 (1.44–2.09)	1.17 (1.04–1.33)	6.35 (5.72–7.05)	1.70 (1.10–2.64)	1.80 (1.42–2.24)	0.72 (0.43–1.22)	0.71 (0.56–0.90)	1.56 (1.02–2.37)
	Yes	0.87 (0.66–1.16)	1.93 (1.57–2.37)	1.04 (0.90–1.19)	5.76 (5.00–6.62)	1.80 (1.17–2.69)	1.85 (1.43–2.39)	0.74 (0.43–1.30)	0.72 (0.56–0.92)	1.62 (1.07–2.44)
Chloramphenicol	No	1.48 (0.85–2.57)	1.58 (0.78–3.18)	0.36 (0.27–0.49)	9.11 (5.90–14.10)	0.69 (0.07–6.66)	1.50 (0.46–4.97)	1.24 (0.27–5.57)	1.92 (0.85–4.36)	3.94 (1.16–13.32)
	Yes	1.20 (0.66–2.18)	0.94 (0.31–2.86)	0.31 (0.20–0.47)	9.15 (5.88–14.23)	0.69 (0.11–4.36)	1.47 (0.52–4.15)	1.16 (0.29–4.62)	1.95 (0.94–4.05)	3.67 (1.18–11.39)
Kanamycin	No	0.77 (0.38–1.56)	2.04 (1.21–3.44)	2.26 (1.53–3.34)	38.69 (25.72–58.20)	10.71 (6.11–18.78)	7.88 (4.26–14.58)	6.59 (2.93–14.80)	5.75 (3.57–9.26)	22.41 (7.98–62.95)
	Yes	0.79 (0.41–1.53)	2.29 (1.52–3.45)	2.58 (1.81–3.69)	42.90 (29.46–62.49)	9.13 (5.02–16.61)	6.54 (3.63–11.79)	5.77 (2.38–13.99)	5.30 (3.29–8.39)	20.12 (6.65–60.84)
Streptomycin	No	1.02 (0.68–1.54)	1.20 (0.77–1.87)	1.49 (1.25–1.76)	5.88 (4.96–6.96)	4.15 (2.94–5.86)	4.69 (3.71–5.93)	2.03 (1.27–3.23)	2.59 (2.06–3.25)	9.59 (6.59–13.95)
	Yes	1.01 (0.67–1.52)	1.20 (0.86–1.68)	1.42 (1.23–1.63)	6.16 (5.27–7.19)	3.85 (2.87–5.16)	4.34 (3.53–5.34)	1.90 (1.22–2.96)	2.56 (2.06–3.19)	8.99 (6.12–13.21)
Sulfisoxazole	No	0.72 (0.58–0.90)	0.91 (0.60–1.38)	1.00 (0.85–1.17)	7.77 (6.60–9.15)	1.14 (0.64–2.01)	1.95 (1.53–2.47)	1.01 (0.66–1.53)	1.68 (1.29–2.17)	5.12 (3.39–7.74)
	Yes	0.70 (0.57–0.86)	0.90 (0.65–1.24)	0.96 (0.83–1.10)	8.13 (6.89–9.59)	1.05 (0.56–1.96)	1.81 (1.45–2.27)	0.95 (0.61–1.46)	1.64 (1.28–2.11)	4.83 (3.20–7.28)
Tetracycline	No	1.1 (0.85–1.42)	0.99 (0.67–1.48)	1.68 (1.50–1.88)	256.73 (167.30–393.98)	25.71 (14.11–46.86)	14.15 (10.59–18.20)	14.53 (9.39–22.48)	20.71 (13.76–31.19)	28.11 (18.92–41.76)
	Yes	1.14 (0.90–1.43)	1.01 (0.71–1.43)	2.15 (1.81–2.56)	344.35 (281.05–421.91)	37.34 (16.94–82.28)	19.22 (12.97–28.47)	18.94 (11.46–31.29)	22.14 (14.33–34.21)	41.16 (25.45–66.55)
Trimethoprim-sulfamethoxazole	No	0.86 (0.56–1.32)	1.17 (0.77–1.80)	1.52 (1.27–1.82)	0.28 (0.24–0.33)	0.26 (0.05–1.37)	0.15 (0.06–0.37)	0.10 (0.01–0.62)	0.09 (0.06–0.14)	0.24 (0.12–0.49)
	Yes	0.83 (0.55–1.24)	1.09 (0.78–1.53)	1.37 (1.17–1.60)	0.31 (0.25–0.39)	0.20 (0.02–1.69)	0.11 (0.03–0.40)	0.07 (0.01–0.57)	0.09 (0.05–0.14)	0.19 (0.07–0.50)

[a] Multivariate resistance phenotypes of commensal *E. coli* isolates from human wastewater and swine fecal samples. Odds ratios (ORs) and 95% confidence intervals (CI) of *E. coli* isolates are presented in contrast to those of host species and human vocation and swine production groups. Isolates are compared across all unit locations and seasons.

[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).

taminated with feces, and the gut contents of the killed pigs that contain higher levels of AR bacteria than those of the non-swine workers and swine workers. Nijsten et al. (12) reported no significant differences between the levels of resistance among the *E. coli* isolates of abattoir workers with or without direct contact with pig fecal contents or pig carcasses.

The adjusted ORs of resistance among pork consumer *E. coli* isolates were lower for five antimicrobial agents than those of the non-swine worker/nonconsumer group. In general, the consumption of food (e.g., pork) from unknown sources may have increased the risk of AR bacteria in the "negative" control group compared that of the pork consumers within the study system (the imported pork trim had a very low *E. coli* prevalence [7.5%]). In addition, there may be differences, albeit unknown to the researchers, in the antibiotic consumption patterns in this population.

In general, the isolation (i.e., purchased) boars showed higher levels of resistance than swine-rearing and slaughtered pigs for ampicillin, chloramphenicol, kanamycin, sulfisoxazole, and tetracycline. In contrast, nursery piglets showed higher levels of resistance than quarantined boars, other swine-rearing, and slaughtered pigs for cefoxitin, ceftiofur, gentamicin, and streptomycin. The quarantined (purchased) boars' higher resistance levels may be attributed to unknown but likely higher historical antimicrobial use within the outside purebred multiplier units. Furthermore, in our study population, nursery piglets received larger amounts of injectable antimicrobial agents than did the other swine production groups (data not shown).

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 likely introduced to the system from the outside, through imported pork. Moreover, all of this trim was processed into breakfast sausage before it was shipped into the system. To the best of our knowledge, there are no comparable literature data for AR *E. coli* levels in pork trim or pork fat.

There was a high seasonal variability, without an apparent seasonal trend, observed among swine and human samples over the 3-year period, for both the individual antimicrobial agent resistance and the multidrug resistance phenotypes. We attempted a time-series analysis to determine the seasonal trend in our longitudinal data. The time-series models did not fit the data well, and that is likely because (i) our data were binary in nature, which made it difficult to analyze using time-series models (time-series models best explain the trends associated with time for continuous data); and (ii) our longitudinal data structure consisted of multiple *E. coli* bacteria isolated from multiple locations within each unit, measured repeatedly at each time point (season). On the other hand, we have ongoing analyses to determine whether the likely source of the seasonal variability is attributable to seasonal differences in historical and concurrent antimicrobial use in swine and human populations or to other risk factors related to management and selective bacterial survival at different times of the 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. Based on an expected median of just two distinct phenotypes and comparisons to that in the literature, e.g., Berge et al. (5) reported a mean of 1.8 phenotypes per five *E. coli* colonies from dairy calf fecal samples, with a total of 5,366 isolates evaluated, the additional resources out of a finite budget consumed by evaluating phenotypes for 5 isolates per sample instead of one, and our much greater interest in assessing resistance levels longitudinally over the 3-year period, we elected instead to assess 1 isolate per sample to test for antimicrobial susceptibility. This ensured statistically that there was no within-sample monthly clustering of results, especially considering the very high intracluster correlation on each plate.

Semiquantitative results (i.e., MICs) are important in order to monitor small shifts in susceptibility and resistance at the population level. However, the MIC data with 2-fold differences in antibiotic concentration per dilution are difficult to analyze statistically and interpret in multivariable models; there were inconsistent numbers of dilution across the 15 antimicrobial agents, and these differences were truly not the subject of this study.

We enriched our human wastewater samples prior to plating, whereas we did not do so for swine feces. The only way in which enrichment of the human wastewater samples and not the swine fecal samples could potentially cause a differential misclassification bias is if resistant (or susceptible) strains of *E. coli* were favored by the enrichment step contained within the wastewater process, but not the swine fecal culture process. The enrichment step for wastewater samples could potentially have changed the composition of resistant and susceptible *E. coli* isolates from wastewater samples and therefore the sample from the enrichment broth that was then plated on the CHROMagar. For example, (i) *E. coli* bacteria might have shed resistance plasmids in the enrichment broth, which would have resulted in an underestimation of the levels of resistance compared to that of the raw wastewater sample; (ii) in a competitive broth (such as the enrichment step), less-fit resistant bacteria may grow slower than susceptible bacteria, hence decreasing the probability of plating and selecting a resistant isolate. These arguments are, in part, based on published results from in vitro noncompetitive and competitive studies. However, it is possible that many other factors affect bacterial growth and competition in a competitive culture (the enrichment step) and possibly result in an increased probability of selecting a resistant *E. coli* isolate, or have no effect at all. In other words, it is impossible to determine the direction of the bias associated with the enrichment step. Hence, in this study, the measures of association may be biased away from or toward the null; that is, the measure of association may be either over- or underestimated when outcomes from swine are compared with those of human populations.

Adjusting for the dependence in the multiple binary outcomes (i.e., multivariate phenotypes) using the SAS macro had a variable effect on the OR values (i.e., ranging from 3 to 28% increase or decrease) and their CIs compared to that of unadjusted ORs and their CIs. In general, there was not a dramatic change in the OR direction of effect (e.g., <1 to >1 or vice versa) when adjusted for multiple binary outcomes. However, the CI for slaughter plant workers included 1 (null value) when adjusted for multiple binary outcomes, indicating that the OR for ampicillin resistance became statistically nonsignificant after the adjustment compared to that of non-swine workers.

There are few or no published works that adjust for the dependence among multiple binary AR outcome data. This is clearly important, especially since several of the antimicrobial agents on the NARMS panels arise from the same class of drugs; that is, we would expect that there should be biological dependence (both pharmacologic effect, as well as genetic linkages in the bacteria) that must be accounted for.

In conclusion, this is the first major longitudinal study conducted to assess the risk of carriage of AR bacteria due to human occupational and consumption exposure to swine in a multisite, vertically integrated agri-food system. The study design and sample collection strategy complements the existing related AR research that has addressed the risk of resistant bacterial transmission to humans as a result of direct contact with animals. In this longitudinal study (over the 3-year period), occupational exposure of the slaughter plant workers to bacteria appeared to be associated with higher cefoxitin resistance and multidrug resistance than that of non-swine workers. The highest *E. coli* resistance prevalence for tetracycline was observed with slaughter plant workers compared to that of the other human vocation cohorts, though the differences were of marginal statistical significance. This finding might be attributed to the higher occupational exposure of slaughter plant workers to AR bacteria than that of non-swine workers. In general, (i) the swine *E. coli* isolates across all units had higher levels of resistance than those of humans, and (ii) the swine production group-resistant isolates differed significantly, with the highest levels found in purchased boars, breeding boars, and nursery piglets. Adjusting for the dependence within multivariate phenotypes, using the multivariate model of correlated dependence had variable effects on the ORs and their CIs before adjustment. Seasonal effect was highly variable over the 3-year study period. An ongoing analysis is being conducted to evaluate the relationship between AR seasonal variability and the recent and concurrent use of antimicrobial agents in both host species.

ACKNOWLEDGMENTS

The longitudinal AR research project was supported in part by the United States Department of Agriculture-Cooperative State Research Education and Extension Service-National Research Initiative, competitive grants program section 32.1 (Epidemiologic Approaches to Food Safety) grant no. 2003-35212-13298.

We thank the administrators of the multisite wastewater and swine operations for their cooperation in conducting this lengthy research. For professional advice and assistance, we thank Howard Gray and the staff of Ace Technologies, Inc. and the Northern Water District. We thank Jacquline Kotzur, Katie Wilson, Autumn Brawley, Jenson Nickel, Shawn Summerlad, Mark Dietz, Kristi Christian, and Keri Norman for their technical assistance in processing samples and performing microbiological assays at our laboratory. Thanks to Rebecca Brian and Mathangi Sivaramakrishnan for providing computer support.

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