

Patterns of Antimicrobial Resistance Among Commensal *Escherichia coli* Isolated from Integrated Multi-Site Housing and Worker Cohorts of Humans and Swine

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ABSTRACT

We examined antimicrobial resistance (AR) phenotypes among commensal *Escherichia coli* isolated from fecal matter of humans and swine housed in a semi-closed and uniquely integrated multi-site farrow-to-plate operation. Aggregate cohorts of humans consisted of (1) "control" groups of consumers, (2) groups of swine workers, and (3) groups of slaughter-plant workers. Analyses of cross-sectional AR data from 472 human and 376 swine isolates are presented. AR phenotypes among these isolates were compared by (1) host species, (2) facility location, (3) facility type, (4) housing (human) or production (swine) cohort, and (5) sample collection period and time of day. There were significant ($p < 0.05$) differences in isolates among host-species with swine uniformly at greater risk for (1) AR to four specific antimicrobials (kanamycin, streptomycin, sulfamethoxazole, tetracycline), and (2) multiple resistance phenotypes ($p < 0.0001$). Facility type and unit location were more often associated with AR differences among swine isolates than among human isolates. Swine production group was significantly associated with AR prevalence ($p < 0.05$) for nine antimicrobials; in general, purchased boars, suckling piglets, weaned piglets, and lactating sows were at higher risk of AR. There was no significant ($p > 0.05$) association of human occupational/consumer cohort with AR prevalence. Several unique AR phenotypes were detected in each of the human- and swine-intake groups. These data establish baseline characteristics for an on-going 3-year longitudinal study designed to further characterize AR phenotype and genotype in this population. Host-, facility-, and cohort-specific data demonstrate that sufficient prevalence differences exist to permit the future quantification of AR transmission, should it occur. Based on these cross-sectional data, occupational exposure to either swine-rearing or swine-slaughter facilities does not appear to be associated with the prevalence of phenotypic resistance among the commensal fecal *E. coli* isolated from this integrated system.

INTRODUCTION

THE USE OF ANTIMICROBIALS provides selective pressure for, and in turn, propagates resistant microbes (Levy, 1992; Witte, 2000). In the presence of an antimicrobial, a resistant bacterium will have a selective advantage and its numbers will be amplified, while susceptible bacteria will likely be inhibited or killed (Helmuth and Protz, 1997; Lipsitch, 2000; O'Brien,

2002). Resistance to two or more classes of antimicrobials among *Escherichia coli* O26, O103, O11, O128, and O145 (Schroeder et al., 2002; White et al., 2002) is now commonplace in isolates from both veterinary and human medicine. Other strains of commensal *E. coli*, which are naturally occurring host-enteric flora, constitute an enormous potential reservoir of resistance genes for pathogenic bacteria (Aarestrup and Wegener, 1999; Anderson et al.,

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2003). The prevalence of resistance in the commensal bacteria of humans and animals is considered to be a good indicator of the selective pressure of antibiotic usage and reflects the potential for resistance in future infections (O'Brien, 2002; Anderson et al., 2003). Commensal *E. coli* are used internationally as indicator bacteria because of their high prevalence in the feces of healthy humans and animals, and because of their ability to harbor several resistance determinants (Teuber, 1999; Witte, 2000; Catry et al., 2003; Shea, 2003).

To date, evidence regarding the risk of animal-to-human transmission of resistant bacteria has been circumstantial—based largely on qualitative cross-sectional data or case series. Many researchers have focused on simplified uni-directional models of transmission (i.e., from animals to humans) that neglect the fundamentally complex ecology of humans interacting with animals in agri-food production and consumption systems (Levin et al., 1997; Barber, 2001). Moreover, these uni-directional models often ignore other potential reservoir species as well as environmental interactions between potential hosts and the pathogen or commensal bacteria of interest (Halling-Sorenson et al., 1998; Barber et al., 2003). Resistant bacteria have been shown to reach the environment via human sewage (Harwood et al., 2001; Huys et al., 2001). It has been shown that swine herds are 11 times more likely to have a high prevalence of *Salmonella* spp. when there is no toilet facility on the premises for human workers (Funk et al., 2001a).

To date, valid and rigorous ecological or epidemiological studies—such as cohort studies—of AR are lacking in the veterinary and human medical literature. That said, investigating potential human health risks associated with the agricultural use of antimicrobials is fraught with difficulties. These include (1) lack of verifiable and quantifiable longitudinal data concerning the actual sources and characteristics of the foods consumed by humans, (2) lack of information regarding human contacts with food animals, food animal products, and other humans, (3) regular in- and out-migration of humans from study areas, and (4) excessive costs and poor compliance of humans associated with long-term follow-up studies. We presently have

available little or no quantitative longitudinal data necessary for useful and reliable risk assessments (Bailar and Travers, 2002; Isaacson and Torrence, 2002; Snary et al., 2004).

The study described herein was designed to characterize the potential inter-host transmission dynamics of AR in an integrated population of animals and humans with minimal outside influence. Our approach was to focus on a carefully monitored and uniquely integrated multi-site farrow-to-plate swine operation. Our objective was to identify baseline AR characteristics of enteric bacteria in animals and humans within the study population—specifically, phenotypic traits that were both unique and common to commensal *E. coli* from each host species.

MATERIALS AND METHODS

Study population

The study population represented a unique arrangement consisting of groups of humans and swine in a semi-closed vertically integrated agri-food system. The population of both humans and swine were housed across multiple unit locations: with eight of these units having both humans and swine present, one “intake” unit with only humans present, one “intake” unit with only swine present, and a single unit with a swine slaughter facility attached.

There was very limited movement of swine into the system and no movement of live swine out of the system (Fig. 1). Almost all replacements were reared within the system, with boars comprising the only outside stock purchased. Purchased pigs were held for several months in quarantine at a single isolated unit (S-INTAKE in Fig. 1) and typically were thereafter sent to a purebred multiplier unit (HS-6 in Fig. 1), which then supplied the other farrowing units with gilts and boars. Swine moved vertically within the system from farrowing units and nurseries (HS-1, 2, 4, 5, and 6 in Fig. 1) to grower and finisher barns (HS-1, 2, 3, 4, 5, 6, 7, and 8 in Fig. 1) and then to slaughter. All swine raised on the farm units were slaughtered, processed, and consumed by humans within the system.

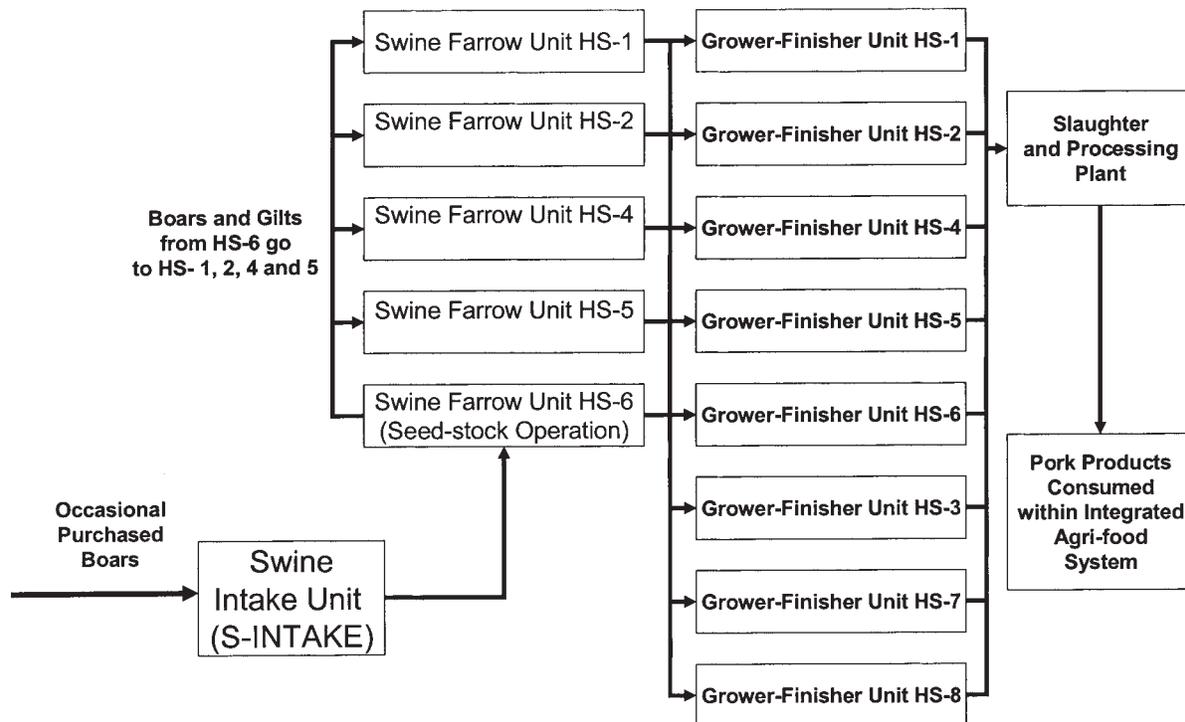


FIG. 1. Schematic diagram illustrating the flows of swine and swine products through the integrated multi-site farrow-to-plate operation.

The human population (Fig. 2) at each of the units with swine-rearing (HS-1, 2, 3, 4, 5, 6, 7, or 8 in Fig. 2) or slaughter (H-SLAUGH in Figure 2) facilities were group-housed in either “worker” or “non-worker” cohorts. The population at the “intake” unit (H-INTAKE in Fig. 2) into the system included only non-workers. All humans were fed centrally prepared meals in a common eating area specific to each cohort group.

Sample collection

From May 2003 through March 2004, monthly composite swine fecal samples, as well as floor-wash, influent, and lagoon samples, were collected from each of the five farrowing units, along with composite samples from each of eight grower-finisher units. Each fecal sample (50 g) was composited from two pen floors—with 5 equal-sized (5 g) samples taken from fresh fecal pats on or near one of the pen’s diagonal axes. Floor-wash, pre-lagoon influent, and lagoon samples were mid-stream (or, mid-lagoon) grab samples of 50 mL each. Fecal samples were collected from the swine quarantine facility (S-IN-

TAKE) on four separate occasions when outside boars were purchased. No other outside purchases of swine occurred during this study. Samples were collected by herd veterinarians and transported on ice to our laboratory.

Human wastewater grab samples were collected by trained facility staff at each of the unit locations on a monthly basis during May–September 2003, and again during February and March 2004. Units were sampled at the closest manhole determined to be draining the lavatory facility for each of the appropriate housing cohorts. These samples were collected at 10:00, 12:00, and 14:00 during the summer and fall of 2003, and once per day during 2004. In addition, a single wastewater treatment plant influent sample (representing the entire unit’s human wastewater) was collected at each collection date. All samples were shipped on ice to our laboratory for further analysis.

Isolation of *E. coli*

All samples were frozen to -70°C upon arrival at the laboratory. Upon thawing, samples were mixed with a sterile inoculating loop and

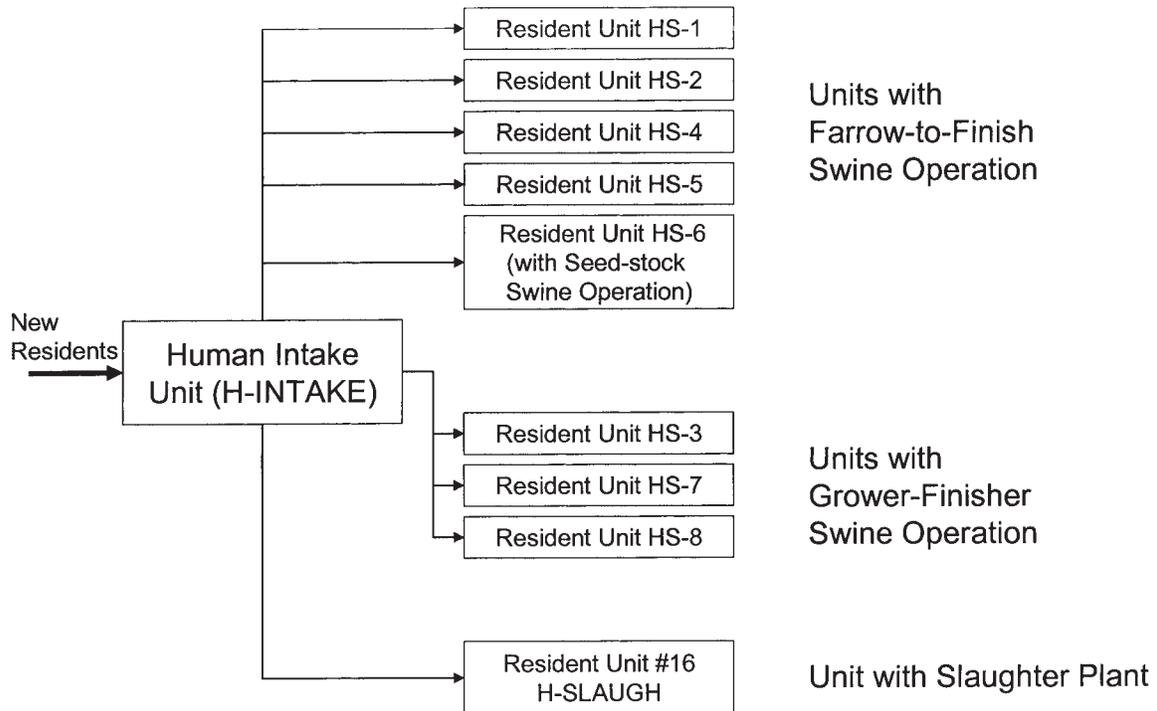


FIG. 2. Schematic diagram illustrating the unit locations that comprise the human populations housed within the integrated multi-site farrow-to-plate operation.

plated onto CHROMagar-*E.coli*TM (DRG International, Mountainside, NJ) and incubated at 37°C for 24 h. Commensal *E. coli* were expressed morphologically as smooth or rough blue colonies. A single smooth blue colony was

selected per plate and re-streaked onto blood agar (5% sheep RBC). Pure culture isolates were verified using biochemical testing (API, bioMerieux, Hazelwood, MO) to ensure correct speciation prior to further testing.

TABLE 1. RANGE OF MICROBROTH DILUTIONS AND BREAKPOINTS FOR DETERMINATION OF *E. coli* RESISTANCE

Antibiotic	Range	Breakpoint
Amikacin	0.5–4	≥64 ^a
Amoxicillin/clavulanic acid	1/0.5–32/16	≥32/16
Ampicillin	1–32	≥32
Cefoxitin	0.5–16	≥16
Ceftiofur	0.12–8	≥8
Ceftriaxone	1–64	≥64
Cephalothin	2–32	≥32
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
Sulfamethoxazole	16–512	≥512
Tetracycline	4–32	≥16
Trimethoprim/sulfamethoxazole	0.12/2.38–4/76	≥4/76

^aThe breakpoint for amikacin falls several dilutions beyond the range provided on the NARMS panels and effectively renders the binary classification scheme (susceptible/resistant) as uninterpretable at ≥4. Amikacin is therefore not considered further in the analyses.

Phenotypic characterization of AR

Phenotypic expression of AR was determined for *E. coli* by measuring the minimum inhibitory concentrations (MIC) against 16 antimicrobials by broth microdilution methods (NCCLS, 1999a). Susceptibility testing was performed on the Sensititre™ automated antimicrobial susceptibility system (Trek Diagnostic Systems, Cleveland, OH) using custom panels designed by the National Antimicrobial Resistance Monitoring System (NARMS) (CDC, 2003). Table 1 identifies the antimicrobials that were used. Breakpoints were determined using NCCLS interpretive standards (NCCLS, 1999b) except for streptomycin for which breakpoints in the NARMS 2001 Annual Report (CDC, 2003) were used. Isolates with intermediate MICs were not considered resistant for our study. The breakpoint for amikacin (≥ 64) fell several dilutions outside the range (0.5–4) on the NARMS panel and is not considered further in this paper. *Enterococcus faecalis* ATCC 29212, *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *E. coli* ATCC 35218 were used as quality control strains for broth microdilution susceptibility testing (NCCLS, 1999b).

Statistical analysis

Data were cross-tabulated for each of the 15 AR phenotypes—as well as the overall total number of resistance phenotypes—with each of the following potential risk factors: (1) host-species, (2) unit location, (3) type of facility, (4) housing cohort (human) or production group (swine), (5) sampling time of day (10:00, 12:00, 14:00), and (6) sampling collection period.

The significance ($p < 0.05$) of differences between the proportion of *E. coli* resistant to each antimicrobial for each risk factor was assessed using either two-tailed Fisher's exact test for 2×2 data, or asymptotic χ^2 likelihood-ratio based methods for $2 \times n$ data (SPSS® for Windows ver. 11.5, SPSS Inc., Chicago, IL). Comparisons between each risk factor and the proportion of *E. coli* resistant to multiple (0–15) antimicrobials were assessed in $m \times n$ tables using asymptotic likelihood-based methods.

Generalized estimating equations (GEE) were used to adjust for the dependence within unit over time whenever the risk factor in ques-

tion was not nested within unit (SAS Institute Inc., 1996; Hardin and Hilbe, 2003). GEE analyses were conducted using SAS® PROC GENMOD (SAS Institute, Cary, NC) by regressing each binary antibiotic resistance outcome on the risk factors in a generalized linear model (GLM) framework. For ordinal response data, the GLM model was constructed using a multinomial distribution and a cumulative logit link function (McCullagh and Nelder, 1989).

RESULTS

There were 848 (472 human, 376 swine) commensal *E. coli* isolated from the fecal and wastewater samples over 14 collection periods. Due to scheduling conflicts, not all units were sampled at each collection period.

Host species

Phenotypic resistance of *E. coli* isolates to each of the 15 interpretable antimicrobials on the NARMS 2003 panel are cross-tabulated in Table 2 by host species. Swine *E. coli* isolates were significantly ($p < 0.05$) more likely to exhibit resistance to kanamycin (odds ratio comparing resistant swine isolates to human ($OR_{sh} = 24.00$, $p = 0.006$), streptomycin ($OR_{sh} = 3.53$, $p = 0.009$), sulfamethoxazole ($OR_{sh} = 15.19$, $p = 0.027$), and tetracycline ($OR_{sh} = 17.65$, $p = 0.001$; Table 2, column 3). There were no swine isolates and only one human isolate resistant to ciprofloxacin. Trimethoprim/sulfamethoxazole was the only antimicrobial with a higher proportion of resistant human isolates than swine isolates ($OR_{sh} = 0.27$, non-significant $p = 0.061$). There were highly significant differences in multiple resistant isolates between host species ($p < 0.0001$; Fig. 3). Fully 50.4% of human isolates were pan-susceptible as compared to 13.6% of swine isolates. While 35.9% of swine *E. coli* isolates were resistant to three or more antimicrobials, 15% of human isolates exhibited these levels of multi-resistance.

Unit location

Resistance data from *E. coli* isolates were also cross-tabulated by unit location (Table 2,

TABLE 2. PHENOTYPIC RESISTANCE OF *E. COLI* ISOLATES FROM HUMAN AND SWINE FECAL SAMPLES

Antimicrobial	Host species (S = swine, H = human)	Odds ratio ^a (p-value) ^b	Percentage resistant <i>E. coli</i> by facility ^c (overall, n = 376 swine (S) isolates and n = 472 human (H) isolates)											p-value ^d			
			H-intake (S = 0) (H = 10)	HS-1 (S = 44) (H = 56)	HS-2 (S = 44) (H = 51)	HS-3 (S = 28) (H = 53)	HS-4 (S = 48) (H = 52)	HS-5 (S = 50) (H = 46)	HS-6 (S = 52) (H = 42)	HS-7 (S = 23) (H = 42)	HS-8 (S = 33) (H = 70)	H-Slaughter (S = 0) (H = 50)	S-Intake (S = 54) (H = 0)				
Amoxicillin/ clavulanic acid	S	1.26 (0.744)	—	9.1	0.0	3.6	8.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.005
	H	—	0.0	8.9	1.9	0.0	1.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	—	0.068
Ampicillin	S	1.55 (0.093)	—	29.5	15.9	14.3	35.4	6.0	21.2	13.0	24.2	—	—	—	—	29.6	0.008
	H	—	10.0	21.4	15.7	18.9	11.5	10.9	9.5	14.3	20.0	12.0	—	—	—	—	0.700
Cefoxitin	S	1.84 (2.406)	—	11.4	0.0	7.1	8.3	4.0	0.0	0.0	0.0	—	—	—	—	0.0	0.003
	H	—	0.0	8.9	3.9	1.9	0.0	0.0	0.0	0.0	0.0	2.0	—	—	—	—	0.027
Ceftiofur	S	2.03 (0.473)	—	6.8	0.0	0.0	8.3	0.0	0.0	0.0	3.0	—	—	—	—	—	0.015
	H	—	0.0	7.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.049
Ceftriaxone	S	9.1 (0.135)	—	11.4	0.0	0.0	0.0	2.0	0.0	0.0	0.0	—	—	—	14.8	<0.0001	
	H	—	0.0	3.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	—	0.476	
Cephalothin	S	1.40 (0.160)	—	27.3	15.9	32.1	39.6	24.0	25.0	17.4	24.2	—	—	—	—	29.6	0.360
	H	—	40.0	35.7	23.5	32.1	32.7	30.4	26.2	38.1	31.4	20.0	—	—	—	—	0.656
Chloramphenicol	S	1.27 (0.499)	—	9.1	0.0	3.6	6.3	2.0	1.9	0.0	9.1	—	—	—	—	3.7	0.188
	H	—	0.0	8.9	0.0	0.0	3.8	0.0	2.4	4.8	4.3	4.0	—	—	—	—	0.085
Ciprofloxacin	S	—	—	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	—	—	—	—	—	—
	H	—	10.0	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	0.553
Gentamicin	S	7.86 (0.129)	—	0.0	4.5	0.0	0.0	0.0	15.4	0.0	0.0	—	—	—	—	—	<0.0001
	H	—	10.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	—	—	—	—	0.336
Kanamycin	S	24.00 (0.006)	—	11.4	15.9	10.7	14.6	10.0	30.8	13.0	6.1	—	—	—	—	—	0.014
	H	—	0.0	0.0	0.0	0.0	0.0	2.2	2.4	4.8	0.0	0.0	0.0	0.0	0.0	—	0.278
Nalidixic acid	S	1.02 (0.978)	—	6.8	2.3	3.6	0.0	2.0	0.0	0.0	0.0	—	—	—	—	—	<0.0001
	H	—	20.0	8.9	9.8	1.9	1.9	2.2	2.4	2.4	4.3	2.0	—	—	—	—	0.190
Streptomycin	S	3.53 (0.009)	—	36.4	43.2	28.6	47.9	22.0	32.7	13.0	12.1	—	—	—	—	—	0.001
	H	—	0.0	14.3	3.9	11.3	9.6	19.6	19.0	9.5	14.3	14.0	—	—	—	—	0.225
Sulfamethoxazole	S	15.19 (0.027)	—	15.9	18.2	14.3	33.3	26.0	19.2	8.7	27.3	—	—	—	—	—	0.268
	H	—	10.0	17.9	5.9	9.4	7.7	8.7	4.8	16.7	18.6	14.0	—	—	—	—	0.224
Tetracycline	S	17.65 (0.001)	—	84.1	75.0	78.6	93.8	64.0	80.8	78.3	69.7	—	—	—	—	—	0.020
	H	—	0.0	14.3	13.7	7.5	21.2	21.7	40.5	11.9	12.9	26.0	—	—	—	—	0.002
Trimethoprim/ sulfamethoxazole	S	0.27 (0.061)	—	0.0	0.0	3.6	2.1	2.0	11.5	0.0	0.0	—	—	—	—	—	0.012
	H	—	10.0	12.5	0.0	9.4	3.8	2.2	2.4	14.3	18.6	8.0	—	—	—	—	0.002

^aOdds-ratios are presented comparing the odds of each phenotype of antimicrobial resistance in swine versus human *E. coli* isolates.

^bp-values are adjusted for the dependence of host species isolate response within each unit location by using the generalized estimating equation (GEE) score statistic (SAR® PROC GENMOD, SAS Institute, Inc., Cary, NC).

^cFacility legend: H-Intake, human isolates from a single intake facility; HS-1 through HS-8, human and swine isolates from eight separate facilities housing both host species; H-Slaughter, human isolates from a single facility with a swine slaughter plant; S-Intake, swine isolates from a single intake/quarantine facility. Note that HS-6 is the sole facility that both receives and rears purebred replacement stock (boars and gilts) supplying the other swine facilities.

^dp-values are based on a likelihood ratio asymptotic two-sided test of the differences in risk across all facilities that were sampled. These data are presented as stratified by host species.

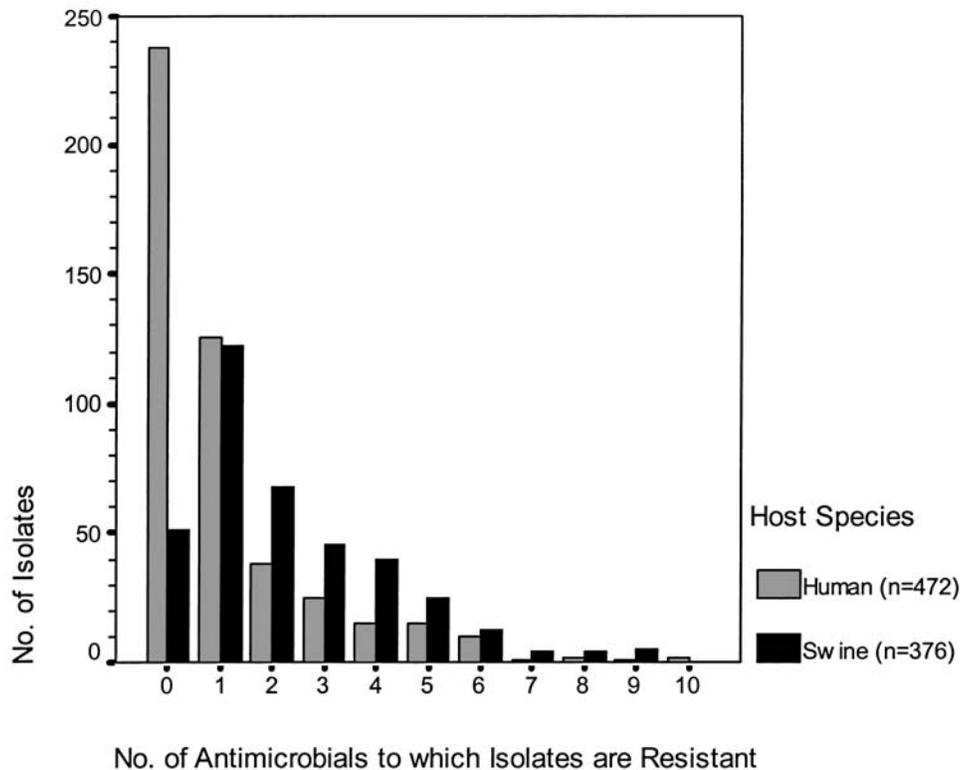


FIG. 3. Frequency bar chart illustrating the distribution of multiple antimicrobial resistance phenotypes (out of 15) exhibited by *E. coli* isolates from human and swine samples.

columns 4–14) and compared within each host species (Table 2, column 15). There were significant differences in resistance among swine isolates across the nine unit locations for 11 antimicrobials: amoxicillin/clavulanic acid ($p = 0.005$), ampicillin ($p = 0.008$), ceftiofur ($p = 0.003$), ceftiofur ($p = 0.015$), ceftriaxone ($p < 0.0001$), gentamicin ($p < 0.0001$), kanamycin ($p = 0.014$), naladixic acid ($p < 0.0001$), streptomycin ($p = 0.001$), tetracycline ($p = 0.020$), and trimethoprim/sulfamethoxazole ($p = 0.012$). For gentamicin, the only units with substantial resistance were S-INTAKE and HS-6. In contrast to swine, across 10 human units, there were significant differences for only four antimicrobials: ceftiofur ($p = 0.027$), ceftiofur ($p = 0.049$), tetracycline ($p = 0.002$), and trimethoprim/sulfamethoxazole ($p = 0.002$).

Facility type

Only those antimicrobials with significant differences of AR by species were further contrasted by facility type (Table 3). Human *E. coli* differed by facility type only for tetracycline ($p =$

0.048), which was highest among all slaughter-plant housing cohorts (26.0%) versus all swine-rearing cohorts (17.2%) and the human-intake (0.0%) cohort. For swine, AR differed only for kanamycin ($p = 0.012$) and streptomycin ($p = 0.046$), with swine-intake showing consistently higher AR than swine-rearing facilities.

Housing cohort

Among human occupational housing cohorts, there were no significant differences ($p > 0.05$) in the risk of *E. coli* phenotypic resistance to any of the 16 antimicrobials (Table 4).

Swine production group differences (Table 5) were apparent for amoxicillin/clavulanic acid ($p = 0.020$), ceftiofur ($p = 0.019$), ceftriaxone ($p = 0.003$), gentamicin ($p < 0.0001$), kanamycin ($p = 0.017$), naladixic acid ($p < 0.0001$), streptomycin ($p < 0.0001$), sulfamethoxazole ($p < 0.0001$), and tetracycline ($p = 0.005$). In general, *E. coli* isolates from purchased boars, piglets, lactating sows, and nursery pigs exhibited the highest prevalence of resistance to all antimicrobials. In contrast,

TABLE 3. *E. COLI* PHENOTYPIC RESISTANCE CONTRASTED BY FACILITY-TYPE FOR ANTIMICROBIALS THAT DIFFERED ($p < 0.05$) BY HOST-SPECIES

Antimicrobial	Host species (S = swine, H = human)	Percentage resistant <i>E. coli</i> by facility-type (overall, n = 376 swine (S) isolates and n = 472 human (H) isolates)				p-value ^a
		Human intake facility (S = 0) (H = 10)	Swine-rearing facility (S = 322) (H = 412)	Swine-slaughter facility (S = 0) (H = 50)	Swine intake facility (S = 54) (H = 0)	
Kanamycin	S	—	14.9	—	29.6	0.012
	H	0.0	1.0	0.0	—	0.579
Streptomycin	S	—	31.4	—	46.3	0.046
	H	0.0	12.6	14.0	—	0.250
Sulfamethoxazole	S	—	21.4	—	24.1	0.797
	H	10.0	11.7	14.0	—	0.878
Tetracycline	S	—	78.3	—	85.2	0.230
	H	0.0	17.2	26.0	—	0.048

^ap-values are based on a likelihood ratio asymptotic two-sided test of the differences in risk of *E. coli* resistance comparing facility types for each host-species.

non-purchased boars, dry sows, grower pigs, and finisher pigs tended to have lower prevalence of AR among *E. coli* isolates.

Time of day

The prevalence of AR among *E. coli* isolated from human wastewater taken at 10:00, 12:00, and 14:00 did not differ significantly ($p > 0.05$)

for any of the 15 antimicrobials (data not shown). There was no difference ($p = 0.623$) in levels of multiple resistance across time of day (data not shown).

Sample collection period

Among human *E. coli* isolates, the prevalence of AR differed significantly ($p < 0.05$) over the

TABLE 4. *E. COLI* PHENOTYPIC RESISTANCE CONTRASTED BY HOUSING GROUP COHORT FOR HUMAN ISOLATES

Antimicrobial	Percentage resistant <i>E. coli</i> by human housing group (n = 455 identified locations)				p-value ^a
	Influent (mixed samples) (n = 147)	Non-swine worker samples (n = 92)	Slaughter-plant worker samples (n = 29)	Swine-worker samples (n = 187)	
Amoxicillin/ clavulanic acid	2.7 11.6	1.1 19.6	3.4 10.3	1.6 15.5	0.745 0.333
Ampicillin	2.7	2.2	3.4	1.1	0.657
Cefoxitin	2.7	2.2	3.4	1.1	0.657
Ceftiofur	1.4	0.0	0.0	1.1	0.467
Ceftriaxone	1.4	0.0	0.0	0.0	0.209
Cephalothin	32.0	38.0	20.7	24.6	0.075
Chloramphenicol	4.8	1.8	3.4	3.2	0.428
Ciprofloxacin	0.0	1.1	0.0	0.0	0.361
Gentamicin	0.7	1.1	3.4	0.0	0.221
Kanamycin	2.0	1.1	0.0	0.0	0.138
Nalidixic acid	4.8	4.3	0.0	4.8	0.435
Streptomycin	13.6	17.4	13.8	9.1	0.241
Sulfamethoxazole	12.9	14.1	17.2	9.6	0.522
Tetracycline	13.6	22.8	27.6	15.5	0.113
Trimethoprim/ sulfamethoxazole	10.2	7.6	10.3	7.5	0.804

^ap-values are based on a likelihood ratio asymptotic two-sided test of the differences in risk between housing cohorts. These p-values are not adjusted for the dependence of responses within unit locations over time.

TABLE 5. *E. COLI* PHENOTYPIC RESISTANCE CONTRASTED BY PRODUCTION GROUP FOR SWINE ISOLATES

<i>Antimicrobial</i>	Percentage resistant <i>E. coli</i> by swine production group (n = 364 identified locations)										p-value ^a
	Boar samples (n = 10)	Dry sow samples (n = 20)	Finisher pig samples (n = 72)	Grower pig samples (n = 50)	Intake boar samples (n = 44)	Lactating sow samples (n = 28)	Lagoon (mixture) samples (n = 18)	Nursery (weaned pig) samples (n = 27)	Floortrash/lagoon influent samples (n = 64)	Piglet samples (n = 30)	
Amoxicillin/ clavulanic acid	0.0	5.0	0.0	0.0	0.0	3.6	0.0	7.4	1.6	13.3	0.020
Ampicillin	36.4	15.0	15.3	24.0	36.4	21.4	11.1	18.5	23.4	26.7	0.298
Cefoxitin	0.0	5.0	0.0	2.0	0.0	7.1	0.0	11.1	3.1	13.3	0.019
Ceftiofur	0.0	5.0	0.0	2.0	0.0	3.6	0.0	7.4	0.0	10.0	0.052
Ceftriaxone	0.0	0.0	0.0	2.0	18.2	7.1	0.0	3.7	1.6	3.3	0.003
Cephalothin	36.4	20.0	27.8	20.0	31.8	35.7	16.7	25.9	25.0	36.7	0.699
Chloramphenicol	0.0	0.0	2.8	6.0	4.5	3.6	0.0	7.4	4.7	6.7	0.740
Ciprofloxacin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	—
Gentamicin	0.0	5.0	0.0	0.0	18.2	0.0	0.0	18.5	1.6	10.0	<0.0001
Kanamycin	0.0	15.0	11.1	10.0	34.1	14.3	16.7	25.9	15.6	30.0	0.017
Nalidixic acid	0.0	0.0	0.0	2.0	25.0	3.6	11.1	0.0	1.6	3.3	<0.0001
Streptomycin	9.1	35.0	20.8	30.0	54.5	28.6	11.1	59.3	28.1	63.3	<0.0001
Sulfamethoxazole	27.3	15.0	13.9	10.0	25.0	17.9	16.7	44.4	18.8	53.3	<0.0001
Tetracycline	100.0	70.0	75.0	92.0	81.8	75.0	50.0	81.5	78.1	90.0	0.005
Trimethoprim/ sulfamethoxazole	0.0	0.0	0.0	2.0	0.0	3.6	5.6	11.1	3.1	3.3	0.171

^ap-values are based on a likelihood ratio asymptotic two-sided test of the differences in risk between swine production groups. These p-values are not adjusted for the dependence of responses within unit locations over time.

entire study period for ampicillin, cephalothin, chloramphenicol, streptomycin, sulphamethoxazole, and tetracycline (data not shown). For swine, AR differed ($p < 0.05$) by sample collection period for ceftriaxone, cephalothin, gentamicin, kanamycin, naladixic acid, streptomycin, sulphamethoxazole, tetracycline, and trimethoprim/sulphamethoxazole.

DISCUSSION

Recent research on AR among swine enteric bacteria has been largely focused on cross-sectional sampling at single or multiple points in the swine production cycle (Mathew et al., 1998; Sunde et al., 1998; Teshager et al., 2000; van den Bogaard et al., 2000; Guerra et al., 2003; Larkin et al., 2004). Other studies have been extended in a longitudinal fashion (Dunlop et al., 1998; Gebreyes et al., 2000; Funk et al., 2001b). The few existing studies that have simultaneously considered both swine and human populations have largely focused on the prevalence of AR bacteria in swine, swine workers, slaughter-plant workers, and/or veterinarians at a single point in time (Nijsten et al., 1994; Bongers et al., 1995; Nijsten et al., 1996; Aubry-Damon et al., 2004) and not on known consumers of pork products from these farms and/or abattoirs.

It is difficult to draw valid conclusions regarding the risk of AR bacteria transmission from food-animals to humans using the studies listed above. First and foremost, assessment of the human exposures is generally lacking. It is widely acknowledged that increased international transportation of both animals and humans (Hakanen et al., 2001), and foods and feedstuffs (Angulo et al., 2000) encourages rapid dissemination of resistance traits worldwide. In- and out-migration of individual humans, multiple-sourced meat products, transportation and dissemination of meat products over long distances, and a myriad of food service handlers make valid "exposure" assessment in the general population nearly impossible. It is the daunting task of assessing the specific dietary and occupational history of human populations that makes AR transmission studies nearly impossible to conduct.

We believe that this paper represents the first published study to directly compare phenotypic AR among *E. coli* isolates not only from livestock and their affiliated occupational cohorts of human workers (both in swine production and slaughter/processing), but also from cohorts known to be consumers of these very same swine products. In our study population, it is also known that there was limited in- and out-migration of both swine and humans to/from this integrated system, and that all pork products being fed to human consumers in the system were processed at the single slaughter facility described in this study. Therefore, this well-characterized study population—with regard to swine and swine product exposure—provided a unique opportunity to test heretofore intractable hypotheses regarding the potential risks of transmission of AR bacteria among livestock and humans.

We found significant ($p < 0.05$) differences in *E. coli* isolates cultivated from the two host-species samples, with swine uniformly at greater risk for (1) AR to four specific antimicrobials (kanamycin, streptomycin, sulfamethoxazole, tetracycline), and (2) for multiple-resistance phenotypes. Pansusceptibility to 16 antimicrobials was common in human *E. coli* isolates and nearly absent in swine. This finding is in agreement with the work of Nijsten et al. (1996), who found significantly higher levels of resistance to oxytetracycline, streptomycin, and sulfamethoxazole in pigs than pig-farmer *E. coli*. In addition, they noted significant ($p < 0.05$) increases in resistance to chloramphenicol, which we did not.

In our study, facility type and unit location were more often associated with AR differences among swine isolates than among human isolates, despite there being known differences among the proportion of the human population at each unit location receiving antimicrobial therapy at any given time (personal communication with senior physician). Variable medical facility load factors were placed on the wastewater plants that drained each unit, which could affect influent AR prevalence. There were also known to be differential usage rates of antimicrobials among the eight swine-rearing facilities in this study (personal communication with herd veterinarians). There is very little in-

formation in the literature with which to compare *E. coli* AR differences among unit locations within integrated swine operations.

Swine production group was significantly associated with AR prevalence ($p < 0.05$) for nine antimicrobials; in general, purchased boars, suckling piglets, weaned piglets, and lactating sows were at higher risk of AR than other groups. Dunlop et al. (1999) attributed much of the variability in *E. coli* AR prevalence within each farm to within-pig and within-pen differences rather than between-pen, within-room, and between-room components. However, they examined individual pig samples, and ours were composites collected across pens within production group. Similar to ours, the study of Mathew et al. (1999) showed differences among production stages as well as by farm type.

Among human samples, there was no significant ($p > 0.05$) association of occupational and/or consumer cohort with AR for any of the 16 antimicrobials. This is in contrast to the findings of Nijsten et al. (1994), who noted significant differences among pig farmers and abattoir workers compared to suburban residents in the Netherlands. In their study, pig farmers showed the highest levels of *E. coli* resistance to amoxicillin, neomycin, oxytetracycline, sulfamethoxazole, and trimethoprim, with abattoir workers second highest and suburban dwellers third. Aubry-Damon et al. (2004) compared multiple enterobacteria from 113 healthy pig farmers to 113 non-farmers and noted significant differences in *E. coli* AR prevalence to streptomycin (relative risk [RR] = 1.40, $p = 0.04$), tetracycline (RR = 2.22, $p < 0.01$), cotrimoxazole (RR = 3.02, $p < 0.01$), and naladixic acid (RR = indeterminate, $p < 0.01$).

In our study, we noted several unique or rare AR phenotypes that were detected in either human- or swine-intake groups. Out of 848 isolates, there was only a single *E. coli*—sampled from the human intake unit—that exhibited resistance to ciprofloxacin. Among human and swine units, resistance to gentamicin was very rare—the exception being samples taken from the swine intake and multiplier units. These findings suggest that certain resistant strains may occasionally be brought into the system. These perceived prevalence gradients and the

absolute presence/absence of resistance must be genotypically confirmed on community DNA using qualitative PCR, and ideally, quantitative PCR before firm conclusions are drawn. This confirmation is needed because of the potential biases associated with cultivation of isolates—i.e., mis-specification of the absence rather than the presence of *E. coli* resistance traits in a host species and/or unit and/or cohort.

While the time of day that samples were collected did not have a significant ($p > 0.05$) association with AR, sample collection period (over several months) was associated with resistance to several antimicrobials ($p < 0.05$). These data demonstrate that sufficient prevalence differences exist not only in cross-section, but also over time, permitting quantification of subsequent AR transmission should it occur.

We selected only a single smooth colony from the primary selective *E. coli* media for each fecal sample processed. While recognizing that there may be other AR genotypes and phenotypes also present on the media, we elected to allocate limited resources across more samples rather than to more isolates within fewer samples. In their study of *E. coli* isolated from dairy calf fecal samples, Berge et al. (2003) sampled five colonies per primary isolate and found a range of 1–4 AR phenotypes with a mean of 1.8 phenotypes. Our experience (unpublished data) has been similar. When comparing phenotypes across risk factors, biased results are not expected to arise directly from selecting fewer isolates per plate, unless the colony selection process is associated (knowingly or not) with the risk factor under consideration (e.g., host species, housing type, facility location). However, estimates of the absolute prevalence of phenotypic AR *E. coli* in any set of samples could be biased if the colony isolation or selection procedure was associated with resistance. For example, if AR phenotype is associated with a fitness disadvantage on selective *in vitro* media, this could occur.

CONCLUSION

This is the first published study to compare antimicrobial resistance characteristics of com-

mensal fecal *E. coli* isolated from aggregated production groups of swine, affiliated occupational cohorts of swine- and slaughter-plant workers, and known consumers of pork products in a multi-site integrated farrow-to-plate system. Our study agreed with previous work suggesting that (1) overall, the level of commensal *E. coli* resistance to one or more antimicrobials is higher among swine than humans, and (2) resistance patterns differ by unit location, farm type, and swine production group. However, unlike other studies, occupational exposure to either swine-rearing or swine-slaughter facilities did not appear to be associated with prevalence of phenotypic resistance among commensal *E. coli*. Further characterization of genotypes and long-term follow-up are needed to assess the transmission of resistant strains across existing prevalence gradients among host-species, unit locations, swine production groups, and occupational/consumer cohorts of humans.

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