Antibiotic-Resistant Enterococci and Fecal Indicators in Surface Water and Groundwater Impacted by a Concentrated Swine Feeding Operation

Amy R. Sapkota,1,2 Frank C. Curriero,1,3 Kristen E. Gibson,1 and Kellogg J. Schwab1

1Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA; 2Maryland Institute for Applied Environmental Health, College of Health and Human Performance, University of Maryland, College Park, Maryland, USA; 3Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA

BACKGROUND: The nontherapeutic use of antibiotics in swine feed can select for antibiotic resistance in swine enteric bacteria. Leaking swine waste storage pits and the land-application of swine manure can result in the dispersion of resistant bacteria to water sources. However, there are few data comparing levels of resistant bacteria in swine manure–impacted water sources versus unaffected sources.

OBJECTIVES: The goal of this study was to analyze surface water and groundwater situated up and down gradient from a swine facility for antibiotic-resistant enterococci and other fecal indicators.

METHODS: Surface water and groundwater samples (n = 28) were collected up and down gradient from a swine facility from 2002 to 2004. Fecal indicators were isolated by membrane filtration, and enterococci (n = 200) were tested for susceptibility to erythromycin, tetracycline, clindamycin, virginiamycin, and vancomycin.

RESULTS: Median concentrations of enterococci, fecal coliforms, and Escherichia coli were 4- to 33-fold higher in down-gradient versus up-gradient surface water and groundwater. Elevated percentages of erythromycin-resistant (p = 0.02) and tetracycline-resistant (p = 0.06) enterococci were detected in down-gradient surface waters, and higher percentages of tetracycline- (p = 0.07) and clindamycin-resistant (p = 0.001) enterococci were detected in down-gradient groundwater.

CONCLUSIONS: We detected elevated levels of fecal indicators and antibiotic-resistant enterococci in water sources situated down gradient from a swine facility compared with up-gradient sources. These findings provide additional evidence that water contaminated with swine manure could contribute to the spread of antibiotic resistance.

KEY WORDS: antibiotic resistance, CAFO, concentrated swine feeding operation, E. coli, enterococci, fecal coliforms, fecal indicators, groundwater, surface water.

RESULTS: Median concentrations of enterococci, fecal coliforms, and Escherichia coli were 4- to 33-fold higher in down-gradient versus up-gradient surface water and groundwater. Elevated percentages of erythromycin-resistant and tetracycline-resistant enterococci were detected in down-gradient surface waters, and higher percentages of tetracycline-resistant and clindamycin-resistant enterococci were detected in down-gradient groundwater.

CONCLUSIONS: We detected elevated levels of fecal indicators and antibiotic-resistant enterococci in water sources situated down gradient from a swine facility compared with up-gradient sources. These findings provide additional evidence that water contaminated with swine manure could contribute to the spread of antibiotic resistance.

KEY WORDS: antibiotic resistance, CAFO, concentrated swine feeding operation, E. coli, enterococci, fecal coliforms, fecal indicators, groundwater, surface water.
well as opportunistic pathogens) that are found in the intestinal tracts of animals and humans and are often used as indicators of fecal contamination in water sources [U.S. Environmental Protection Agency (U.S. EPA) 2000]. The presence of other fecal indicators, including fecal coliforms and *E. coli*, was also investigated in surface water and groundwater samples collected throughout this study.

**Materials and Methods**

**Study site.** This study was conducted around a swine finishing CAFO located in a rural area in the Mid-Atlantic United States (Figure 1). The CAFO is composed of two tunnel-ventilated swine houses, and the full day-to-day capacity of the entire facility is 5,000 hogs. However, throughout the sampling period, approximately 3,000 hogs were present at the facility. Manure wastes from the CAFO are stored in 12-ft deep concrete manure pits that lie on-site (Figure 1) and off-site. At this facility, nontherapeutic levels of antibiotics are both on-site (Figure 1) and off-site. At this facility, nontherapeutic levels of antibiotics are

**Sample collection.** Surface water and groundwater samples were collected during six sampling trips that took place between 2002 and 2004 (Table 1). A total of 15 surface water samples were collected from three locations situated down gradient from the swine CAFO, and a total of 4 surface water samples were recovered from one location situated up gradient from the swine CAFO (Figure 1). As indicated in Figure 1, the down-gradient surface water sampling locations were situated in a stream system that was likely affected by surface water runoff events from the swine CAFO. Sampling locations on two different, connecting tributaries in this stream system were chosen in order to determine the impacts of the swine CAFO on both of these tributaries. Down-gradient surface water samples were collected only when there was adequate flow at a sampling location such that water samples could be collected into 1-L sampling bottles in an upstream motion, midway between the surface and the stream bottom, without disturbing bottom sediment. We were unable to obtain access to an up-gradient surface water sampling location situated within the same stream system because a) we could not penetrate dense and deep thickets that completely surrounded the stream (on accessible property) without making major modifications to existing vegetation; or b) we were not allowed access to personal property farther upstream. Because of these challenges, we identified an up-gradient pond located on accessible property (Figure 1) to serve as an up-gradient surface water control site that was not affected by the swine CAFO.

**Groundwater samples.** Groundwater samples were collected from one drinking water well situated down gradient from the swine CAFO (*n* = 4) and one drinking water well situated up gradient from the swine CAFO (*n* = 5) (Figure 1). Both wells are located in the Piedmont Plateau Province of the Mid-Atlantic United States in an area characterized by unmetamorphosed bedrock composed of red shale. The up-gradient well was constructed in 1990 and is used as a primary source of drinking water by the property owners. It is 250 ft deep and lined with steel casing to a depth of 56 ft. Water is encountered at depths of 185 ft and 228 ft. The down-gradient well is an older well that was used as a primary source of drinking water by the property owners before the neighboring swine CAFO was built. Information on the precise depth and construction of this well was unavailable; however, groundwater on the property is encountered at depths of approximately 90 ft and 132 ft. None of the wells were subject to any disinfection before sampling; at each well, water was flushed for 1 min before groundwater samples were collected.

A manure pit sample was collected directly from the manure pits during one sampling trip in January 2004. All surface water, groundwater, and manure pit samples were collected in 1-L sterile Nalgene Wide Mouth Environmental Sample Bottles (Nalgene, Lima, OH); labeled; and transported back to the laboratory at 4°C. Sample processing took place within 3–6 hr after sample collection.

**Isolation and enumeration of fecal indicators.** *Enterococcus* spp., *E. coli*, and fecal coliforms were isolated from each water sample using standard membrane filtration methods: U.S. Environmental Protection Agency (EPA) Method 1106.1 and Method 1103 (U.S. EPA 2000), and standard method SM 9222D [American Public Health Association (APHA) 1998]. Briefly, 10-fold dilutions of each water sample were prepared (10⁰, 10⁻¹, 10⁻², and 10⁻³), and 10 mL of each dilution were filtered through 0.45-µm, 47-mm mixed cellulose ester filters (Millipore, Billerica, MA), which were placed onto appropriate agar plates. We used mE agar for the detection and enumeration of *Enterococcus* spp., mTEC agar for the detection of *E. coli*, and mFC agar for the detection of fecal coliforms (all from Becton Dickinson, Sparks, MD). Negative control filters and negative control agar plates were included in each membrane filtration analysis. Incubation conditions for the agar plates were as follows: mE plates, 41.5°C for 48 hr; mTEC plates, 35°C for 2 hr followed by 44.5°C for 22 hr; and mFC plates, 44.5°C for 24 hr. After 24 hr, membrane filters from mTEC agar plates were placed in 1.2 mL urea for 5 min; bright yellow colonies were considered presumptive *E. coli*. Blue colonies arising on the mFC agar plates were considered presumptive fecal coliforms. After 48 hr, membrane filters from mE agar plates were plated on esculin iron agar (EIA) plates and incubated at 41.5°C for 20 min. Colonies characteristic of *Enterococcus* spp., ranging from pink to dark red on mE agar and producing a brown to black precipitate on EIA agar, were considered presumptive *Enterococcus* spp.

![Figure 1. Map of study site and sampling locations.](image)

**Figure 1.** Map of study site and sampling locations. Abbreviations: DG GR, down-gradient groundwater sampling location; DG SW 1, first down-gradient surface water sampling location; DG SW 2, second down-gradient surface water sampling location; DG SW 3, third down-gradient surface water sampling location; UG GW, up-gradient groundwater sampling location; UG SW, up-gradient surface water sampling location. Topographic contour lines are given in feet, and contour intervals = 20 vertical ft. Arrows indicate the direction of surface water flow. Topographic data were obtained from a U.S. Geological Survey map of the study area (U.S. Geological Survey 2006).

| Sampling date | UG GW | DG GW | UG SW | Site 1 | Site 2 | Site 3 | Manure pit |
|---------------|-------|-------|-------|--------|--------|--------|------------|
| 29 Sep 2002   |       |       |       | 1      | 1      | 1      | 1          |
| 31 Mar 2003   | 1     | 1     |       | 1      | 1      | 1      | 1          |
| 11 Jun 2003   | 1     | 1     | 1     | 1      | 1      | 1      | 1          |
| 24 Jun 2003   | 1     | 1     | 1     | 1      | 1      | 1      | 1          |
| 30 Jul 2003   | 1     | 1     | 1     | 1      | 1      | 1      | 1          |
| 6 Jan 2004    | 1     | 1     | 1     | 1      | 1      | 1      | 1          |

Abbreviations: DG, down gradient; GW, groundwater; SW, surface water; UG, up gradient.
Identify the type of image: "Natural text". The image contains a document page with text and a table.

The text discusses the detection of Enterococcus spp. in water samples and groundwaters, and the identification of these samples using various tests. The text also includes a table comparing the median and range of concentrations of fecal indicators in up-gradient and down-gradient samples.

### Table 2: Concentrations (CFU/100 mL) of fecal indicators in up-gradient (n = 4) and down-gradient (n = 15) surface water samples and up-gradient (n = 5) and down-gradient (n = 4) groundwater samples collected in the proximity of a swine CAFO.

| Sample type and bacteria | Up-gradient samples [median (range)] | Down-gradient samples [median (range)] | p-Value<sup>a</sup> |
|-------------------------|-------------------------------------|---------------------------------------|---------------------|
| Surface water           |                                     |                                       |                     |
| Enterococcus spp.       | 35 (1–100)                          | 610 (150–4,700)                      | 0.003               |
| E. coli                 | 35 (0–40)                           | 400 (10–2,500)                      | 0.007               |
| Fecal coliforms         | 15 (0–70)                           | 500 (18–2,400)                      | 0.010               |
| Groundwater             |                                     |                                       |                     |
| Enterococcus spp.       | 18 (0–67)                           | 85 (16–140)                         | 0.085               |
| E. coli                 | 0 (0)<sup>c</sup>                   | 11.5 (3–40)                         | 0.007               |
| Fecal coliforms         | 0 (0)<sup>c</sup>                   | 20.5 (3–70)                         | 0.007               |

<sup>a</sup> Median and range summaries are reported to match more consistently with the nonparametric statistical tests performed. <sup>b</sup>P-Values were calculated using the two-sample Wilcoxon rank-sum test. <sup>c</sup>No E. coli or fecal coliforms were detected in these samples on any sampling trip.
comparing rates of clindamycin resistance and virginiamycin resistance were restricted to non-\textit{E. faecalis} isolates. All statistical analyses were performed using Intercooled Stata 7.0 (Stata Corporation, College Station, TX).

**Results**

**Concentrations of fecal indicators.** Median concentrations of \textit{Enterococcus} spp., \textit{E. coli}, and fecal coliforms were 17-, 11-, and 33-fold higher, respectively, in surface waters located down gradient of the swine CAFO compared with surface waters located up gradient of the CAFO; the differences were statistically significant ($p = 0.003$, 0.007, and 0.010, respectively) (Table 2). Likewise, median concentrations of \textit{Enterococcus} spp., \textit{E. coli}, and fecal coliforms found in manure pit samples were 5.2 $\times$ 10\textsuperscript{5} CFUs/100 mL, 1.0 $\times$ 10\textsuperscript{6} CFUs/100 mL, and 8.8 $\times$ 10\textsuperscript{8} CFUs/100 mL, respectively.

\textit{Enterococcus} spp. isolated from water and manure pit samples. A variety of \textit{Enterococcus} spp. was identified in groundwater, surface water, and manure pit samples (Table 3). \textit{E. faecalis} was the predominant species isolated from all sample types, representing 67% of all \textit{Enterococcus} spp. that were analyzed for antibiotic susceptibility in this study. For 29 (14.5%) of the \textit{Enterococcus} spp., results from the standard biochemical identification tests were not completely consistent with known species of enterococci. These isolates could only be identified to the genus level and are listed as “other \textit{Enterococcus} spp.” in Table 3.

**Antibiotic resistance.** Overall, higher erythromycin and tetracycline MICs were detected among \textit{Enterococcus} spp. (\textit{E. faecalis} and non-\textit{E. faecalis}) recovered from down-gradient groundwater and surface water samples compared with up-gradient groundwater and surface water samples (Table 4). For example, erythromycin MIC\textsubscript{90} (MIC required to inhibit the growth of 90% of organisms) for \textit{Enterococcus} spp. recovered from down-gradient groundwater and surface water samples were at least 4-fold and 128-fold higher, respectively, than that of isolates recovered from up-gradient groundwater and surface water samples. These data suggest that down-gradient surface water and groundwater sources are contaminated with \textit{Enterococcus} spp. that express higher levels of erythromycin and tetracycline resistance. The highest erythromycin and tetracycline MICs were observed among \textit{Enterococcus} spp. recovered from manure pits, where erythromycin and tetracycline MIC\textsubscript{90} were > 256 $\mu$g/mL and 179.2 $\mu$g/mL, respectively (Table 4). In contrast, MICs for virginiamycin, a drug that has never been approved for use in U.S. swine production, were generally below the CLSI vancomycin resistance breakpoint of $\geq$ 32 $\mu$g/mL (CLSI 2002) among \textit{Enterococcus} spp. recovered from all sample types. The exceptions were isolates recovered from up-gradient groundwater samples, which exhibited elevated vancomycin MICs (Table 4).

Similar to the findings for erythromycin and tetracycline, higher clindamycin and virginiamycin MICs were observed among non-\textit{E. faecalis} isolates recovered from down-gradient groundwater and surface water samples compared with up-gradient groundwater and surface water samples (Table 5). For instance, clindamycin MIC\textsubscript{90}, for non-\textit{E. faecalis} isolated from down-gradient groundwater and surface water samples were at least 2,133-fold and 2-fold higher, respectively, than that of non-\textit{E. faecalis} recovered from up-gradient groundwater and surface water samples. The highest clindamycin and virginiamycin MICs were observed among isolates recovered from manure pits (Table 5). As anticipated, clindamycin and virginiamycin MICs among \textit{E. faecalis}—which have been shown to be intrinsically resistant to both of these antibiotics (Singh and Murray 2005)—were similar among isolates recovered from all sample types, except in the case of \textit{E. faecalis} recovered from manure pits. These isolates exhibited higher levels of both clindamycin and virginiamycin resistance (Table 5).

In comparing the percentage of antibiotic-resistant \textit{Enterococcus} spp. present in up-gradient versus down-gradient surface water samples, higher percentages of erythromycin-, tetracycline-, virginiamycin-, and vancomycin-resistant isolates were observed in down-gradient versus up-gradient surface waters (Table 6). In contrast, we observed a higher percentage of clindamycin-resistant isolates in up-gradient surface water samples. However, using Fisher’s exact test, we found that only the elevated percentage of erythromycin-resistant isolates found in down-gradient surface water samples was statistically significant ($p = 0.02$) (Table 6). The higher percentage of tetracycline-resistant isolates observed in down-gradient surface water samples was marginally significant ($p = 0.06$) (Table 6).

In groundwater samples, higher percentages of tetracycline- and clindamycin-resistant \textit{Enterococcus} spp. were observed in down-gradient versus up-gradient groundwater samples (Table 6). The elevated percentage of clindamycin-resistant isolates in down-gradient groundwater samples was statistically significant ($p < 0.001$), whereas the higher percentage of tetracycline-resistant isolates in down-gradient groundwater samples was marginally significant ($p = 0.07$) (Table 6). Conversely, higher percentages of erythromycin- and vancomycin-resistant \textit{Enterococcus} spp. were observed in up-gradient versus down-gradient groundwater samples, and the differences in erythromycin resistance were statistically significant ($p < 0.001$).

**Discussion**

In this study we investigated surface water and groundwater located up gradient and down gradient of a swine CAFO for the presence of fecal indicators (\textit{Enterococcus} spp., \textit{E. coli}, and fecal coliforms) and antibiotic-resistant enterococci. Findings indicate that surface waters and groundwater located down gradient of the swine CAFO are contaminated with significantly higher levels of \textit{Enterococcus} spp., \textit{E. coli}, and fecal coliforms compared with surface water and groundwater located up gradient of the swine CAFO (Table 2). The groundwater data are in agreement with two previous studies that examined groundwater wells situated near large-scale swine facilities (Anderson and Sobsey 2006; Krapac et al. 2002). Anderson and Sobsey (2006) detected \textit{E. coli} at a range of 0.5–32.7 CFU/100 mL in groundwater samples collected at two large-scale swine facilities in North Carolina. Krapac et al. (2002) detected fecal coliforms at a maximum concentration of 7 CFU/100 mL in shallow groundwater samples collected at a swine finishing facility in Illinois. In addition, Krapac et al.

Table 4. MIC data (µg/mL) for erythromycin, tetracycline, and vancomycin among \textit{Enterococcus} spp. isolated from groundwater, surface water, or manure pits.

| \textit{Enterococcus} spp. source | Erythromycin\textsuperscript{a} | Tetracycline\textsuperscript{a} | Vancomycin\textsuperscript{a} |
|----------------------------------|-------------------------------|-------------------------------|-----------------------------|
|                                  | MIC\textsubscript{90}         | MIC\textsubscript{90}         | MIC\textsubscript{90}       |
| Up-gradient groundwater (n = 30) | 16                            | 80.8                          | 1–128                       |
| Down-gradient groundwater (n = 26) | 2 > 256                       | < 0.5–256                     | 2 > 1–64                    |
| Up-gradient surface water (n = 22) | 1 2                           | 0.5–4                         | < 1 108.8                   |
| Down-gradient surface water (n = 107) | 2 > 256                     | > 0.5–256                     | < 1 128.8                   |
| Manure pit (n = 15) | 2 > 256 | 180.2 | 128 179.2 |

\textsuperscript{a}MIC\textsubscript{90} is the MIC required to inhibit the growth of 90% of organisms.

\textsuperscript{a}CLSI resistance breakpoints are as follows: erythromycin, ≥ 8 µg/mL; tetracycline, ≥ 16 µg/mL; vancomycin, ≥ 32 µg/mL (CLSI 2002).
(2002) detected fecal streptococcus in more groundwater samples and at higher concentrations than fecal coliforms. Similarly, we identified E. coli and fecal coliforms in down-gradient groundwater samples at ranges of 3–40 CFU/100 mL and 3–70 CFU/100 mL, respectively, and Enterococcus spp. (members of the fecal streptococcus group) were consistently detected at higher concentrations than fecal coliforms (Table 2). To our knowledge, the surface water data presented here are the first data to compare levels of fecal indicators in up-gradient versus down-gradient surface waters located in the proximity of a swine CAFO.

The presence of Enterococcus spp., E. coli, and fecal coliforms in rural surface water and groundwater sources impacted by swine CAFOs may pose health risks to people who either recreate in contaminated surface waters or use the groundwater as a drinking water source. Concentrations of Enterococcus spp. and E. coli in down-gradient surface water samples collected in this study were consistently in excess of the following U.S. EPA bacterial water quality standards for recreational fresh waters: Enterococcus spp., 33 CFU/100 mL; and E. coli, 126 CFU/100 mL (U.S. EPA 2003). Throughout the sampling period for this study, young children were observed swimming and playing in surface waters located within 500 m down gradient of the swine CAFO; these children could have been exposed to elevated concentrations of Enterococcus spp., E. coli, and other more harmful microorganisms that may have been present. In addition, if the down-gradient private well tested in this study was part of a public drinking-water-system testing program, it consistently would be in violation of current maximum contaminant level standards for total coliforms (including fecal coliforms and E. coli) (U.S. EPA 2002). On each sampling trip, this down-gradient well tested positive for both fecal coliforms and E. coli. Before the swine CAFO began production, the owners of this well relied on it as their drinking water well located 400 m down gradient from the facility. After the facility reached a full working capacity of 5,000 hogs, the owners told us that they had their well tested by an independent, certified laboratory and the water was subsequently deemed nonpotable.

The results of this study also emphasize that human health risks associated with exposures to surface water and groundwater situated down-gradient of swine CAFOs could be exacerbated by the presence of antibiotic-resistant bacteria. Overall findings indicate that Enterococcus spp. recovered from down-gradient surface water and groundwater samples express higher levels of resistance (higher MICs) to antibiotics that are commonly used in both swine production and human clinical medicine (erythromycin, tetracycline, clindamycin, and virginiamycin) compared with Enterococcus spp. recovered from up-gradient surface water and groundwater samples (Tables 4 and 5). In contrast, Enterococcus spp. recovered from all sample types (down-gradient water samples, up-gradient water samples, and manure samples) were, in general, similarly susceptible to vancomycin (Table 4), a drug that has never been approved for use in U.S. swine production.

The patterns of antibiotic resistance observed in Enterococcus spp. recovered from down-gradient surface water and groundwater samples were similar to those observed in isolates recovered from manure pit samples, particularly resistance patterns associated with erythromycin, tetracycline, and clindamycin (Tables 4 and 5). We also have reported similar patterns of erythromycin, tetracycline, and clindamycin resistance among Enterococcus spp. recovered from indoor air samples collected within the same swine CAFO during the same sampling period (Chapin et al. 2005). These data support previous findings of Chee-Sanford et al. (2001) showing that the movement of resistant bacteria and resistance determinants from swine CAFOs into the environment can be extensive. Chee-Sanford et al. (2001) found a high occurrence of tetracycline resistance determinants in groundwater wells located close to swine lagoons; however, they also detected one resistance determinant in a well situated over 250 m downstream of one of the lagoons. In the present study, antibiotic-resistant Enterococcus spp. were detected in a drinking water well located 400 m down gradient of a swine CAFO, as well as in surface water situated 300 m down gradient from the facility (Figure 1). The presence of resistant bacteria in both drinking water and surface water sources contaminated by swine CAFOs could contribute to the spread and persistence of both resistant bacteria and antibiotic resistance determinants in humans and the environment.

However, in rural environments, swine CAFOs are not the only potential sources of antibiotic-resistant bacteria. Other sources could include poultry farms, dairy farms, and human sources such as leaking septic tanks and land-applied biosolids. In the present

### Table 5. MIC data (µg/mL) for clindamycin and virginiamycin among E. faecalis and non–E. faecalis isolated from groundwater, surface water, or manure pits

| Enterococcus spp. source | Clindamycin\(^a\) | Virginiamycin\(^a\) |
|-------------------------|------------------|-------------------|
| Up-gradient groundwater | MIC\(_{50}\) | MIC\(_{90}\) | MIC range | MIC\(_{50}\) | MIC\(_{90}\) | MIC range |
| E. faecalis (n = 12) | 8 | 16 | 0.06–16 | 1 | 1 | 0.5–1 |
| Non–E. faecalis (n = 18) | <0.03 | 0.06 | <0.03–0.06 | 0.13 | 0.13 | 0.05–0.13 |
| Down-gradient groundwater | 8 | 28.8 | 0.5–128 | 1 | 2 | 1–4 |
| E. faecalis (n = 21) | 8 | >128 | 4–128 | 0.5 | 1 | 0.5–1 |
| Non–E. faecalis (n = 5) | 128 | >256 | >256 | 1 | 2 | 1–16 |
| Non–E. faecalis (n = 8) | 64 | >128 | 4–128 | 0.5 | 1 | 0.5–2 |
| Non–E. faecalis (n = 8) | 128 | >256 | 4–128 | 0.5 | 1 | 0.5–2 |
| Manure pit | 192 | >256 | >256 | 1 | 2 | 1–32 |
| E. faecalis (n = 7) | 128 | >256 | 4–256 | 1 | 2 | 1–16 |
| Non–E. faecalis (n = 8) | 128 | >256 | >256 | 1 | 2 | 0.5–32 |

MIC\(_{50}\) and MIC\(_{90}\) required to inhibit the growth of 50% of organisms.

\(^a\)CLSI resistance breakpoint for clindamycin and virginiamycin is ≥ 4 µg/mL (CLSI 2002).

### Table 6. Percentage of antibiotic-resistant Enterococcus spp. in up-gradient (n = 4) versus down-gradient (n = 15) surface water samples and up-gradient (n = 5) versus down-gradient (n = 4) groundwater samples.

| Sample type and antibiotic | Percent resistant |
|----------------------------|-------------------|
|                            | Up-gradient samples | Down-gradient samples | p-Value |
| **Surface water**          |                   |                    |        |
| Erythromycin                | 0                 | 18                 | 0.02   |
| Tetracycline                | 14                | 33                 | 0.06   |
| Clindamycin\(^a\)          | 100               | 89                 | 0.76   |
| Virginiamycin\(^a\)        | 0                 | 23                 | 0.17   |
| Vancomycin                  | 0                 | 1                  | 0.83   |
| **Groundwater**            |                   |                    |        |
| Erythromycin                | 67                | 20                 | <0.001 |
| Tetracycline                | 3                 | 19                 | 0.07   |
| Clindamycin\(^a\)          | 100               | <100               | <0.001 |
| Virginiamycin\(^a\)        | 0                 | 0                  | —\(^b\)  |
| Vancomycin                  | 10                | 0                  | 0.15   |

p-Values were calculated using one-sided Fisher’s exact tests.

\(^a\)Analyses for clindamycin and virginiamycin resistance were restricted to non–E. faecalis isolates. \(^b\)No p-value could be calculated due to zero counts of virginiamycin-resistant isolates in both sample types.
study, an unexpected finding was that up-gradient groundwater samples that were not impacted by the swine CAFO contained significantly higher percentages of erythromycin-resistant Enterococcus spp. compared with down-gradient groundwater samples (Table 6). The levels of erythromycin resistance (MICs) in these isolates were not as high as those observed in Enterococcus spp. recovered from down-gradient groundwater samples and manure pit samples (Table 4); however, lower-level erythromycin-resistant Enterococcus spp. were still present in significant numbers. After sampling was completed, the owners of this up-gradient well informed us that they had experienced problems with their septic tank and field in the past, and perhaps this may have contributed to the presence of erythromycin-resistant Enterococcus spp. in their well. However, the role of possible contamination from their septic tank was not confirmed. Similarly, we found a slightly higher percentage of clindamycin-resistant non-E. faecalis in up-gradient surface water samples compared with down-gradient surface water samples (Table 6). Although the difference was not statistically significant and the levels of clindamycin resistance observed in these isolates were lower than those of non-E. faecalis recovered from down-gradient surface water samples and manure samples (Table 5), the presence of resistant non-E. faecalis in up-gradient surface water suggests that additional sources of resistant bacteria may exist in this environment. These sources could include human septic tank, companion animals, wild animals, and migratory waterfowl such as Canada geese (Middleton and Ambrose 2005; Sayah et al. 2005). These findings point to the challenges of identifying pristine, uncontaminated control sites for field studies of water sources located in rural settings, where a variety of agricultural and other human and animal activities can introduce pollutants into the surrounding environment.

Limitations of this study concern sample size and antibiotic usage data. A larger sample size would have provided more statistical power to detect differences in percentages of antibiotic-resistant bacteria present in up-gradient versus down-gradient water samples. Additional samples also would have allowed for statistical analyses regarding seasonal variations in water quality. Beyond sample size, this study would have been enhanced if we had been able to obtain specific antibiotic usage data from the swine grower. Unfortunately, the grower did not have this information because the feed used in this facility was premixed and delivered to the swine CAFO by the contracted integrator, which had deemed antibiotic usage data proprietary information. Instead, we used general FDA data describing the types of antibiotics approved for use in U.S. swine production (FDA 2004) to determine which antibiotics to test in this study. In future studies, we plan to improve the sample design (including sample size) so that statistical analyses can be used to explore spatial and temporal variation in antibiotic-resistant bacteria as it relates to surrounding swine CAFOs. However, the difficulties in obtaining specific antibiotic usage data from swine growers could continue to be a challenge for environmental health researchers in the absence of federal and/or state regulations that require growers or integrators to report these data.

Conclusions

We observed high levels of erythromycin, tetracycline, and clindamycin resistance in Enterococcus spp. recovered from surface water and groundwater situated down gradient from a swine CAFO compared with surface water and groundwater located up gradient of the facility. Significantly elevated concentrations of all three fecal indicators tested in this study were also observed in down-gradient surface water and groundwater samples compared with up-gradient surface water and groundwater samples. Although the specific source or sources of these contaminants was not definitively determined, it is likely that swine manure pit leakage or runoff from swine manure-applyed fields (Thurston-Enriquez et al. 2005) contributed to these findings. Swine manure management practices, as well as swine feeding practices such as the administration of nontherapeutic levels of antibiotics in swine feeds, continue to pose both environmental and public health challenges, particularly in the immediate environment of swine CAFOs, where vast amounts of swine manure are produced and applied to agricultural fields.

References

Aarestrup FM, Kruse H, Tast E, Hammerum AM, Jensen LB. 2000. Associations between the use of antimicrobial agents for growth promotion and the occurrence of resistance among Enterococcus faecium from broilers and pigs in Denmark, Finland, and Norway. Microb Drug Resist 6:63–70.

Anderson ME, Sobsey MD. 2006. Detection and occurrence of antimicrobially resistant E. coli in groundwater on or near swine farms in eastern North Carolina. Water Sci Tech 54:211–218.

APHA. 1984. Standard Methods for the Examination of Water and Wastewater. 19th ed. Washington, DC:American Public Health Association.

Bagger G, Madsen M, Christensen J, Aarestrup FM. 1997. Avoparcin used as a growth promoter is associated with the occurrence of vancomycin-resistant Enterococcus faecium on Danish poultry and pig farms. Prev Vet Med 31:95–112.

Campagnolo ER, Johnson KR, Karpati A, Rubin CS, Kolpin DW, Meyers MT, et al. 2002. Antimicrobial residues in animal waste and water resources proximal to large-scale swine and poultry feeding operations. Sci Total Environ 299:89–95.

Chapin A, Rule A, Gibson K, Buckley T, Schwab K. 2005. Airborne multidrug-resistant bacteria isolated from swine manure. Environ Health Perspect 113:137–142.

Chee-Sanford JC, Aminov RJ, Krapac LJ, Garrigues-Jeanjean N, Mackie RL. 2001. Occurrence and diversity of tetracycline resistance genes in lagoons and groundwater underlying two swine production facilities. Appl Environ Microbiol 67:1484–1502.

CLSI (Clinical and Laboratory Standards Institute). 2002. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard—Second edition. Wayne, PA:National Committee for Clinical Laboratory Standards.

FDA (U.S. Food and Drug Administration). 2004. FDA Approved Animal Drug Products. Blacksburg, VA:Drug Information Laboratory, Virginia/Maryland Regional College of Veterinary Medicine.

Haack BJ, Andrews RE Jr. 2000. Isolation of Tn916-like conjugal elements from swine lot effluent. Can J Microbiol 46:542–549.

Jongbloed AW, Lenis NP. 1998. Environmental concerns about animal manure. J Anim Sci 76:2641–2648.

Krapac LJ, De Y, W2, Roy WR, Smyth CA, Storment E, Sargent SL et al. 2002. Impacts of swine manure pits on groundwater quality. Environ Qual 120:473–492.

Mellon M, Benbrook C, Benbrook KL. 2001. Hitting it! Estimates of antimicrobial abuse in livestock. Available: www.ucosula.org/food_and_environment/antibiotics_and_food/hitting-it_estimates-of-antimicrobial-abuse-in-livestock.html [accessed 15 September 2006].

Middleton JH, Ambrose A. 2005. Enumeration and antibiotic resistance patterns of fecal indicator organisms isolated from migratory Canada geese (Branta canadensis). J Wildl Dis 41:334–341.

Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH. 2003. Manual of Clinical Microbiology. 7th ed. Washington, DC:American Society for Microbiology Press.

Parveen S, Lukasik J, Scott TM, Tampil ML, Portier KM, Shepard S, et al. 2006. Geographical variation in antibiotic resistance profiles of Escherichia coli isolated from swine, poultry, beef, and dairy cattle farm water retention ponds in Florida. J Appl Microbiol 100:1050–1057.

Sayah RS, Kaneene JB, Johnson Y, Miller R. 2005. Patterns of antimicrobial resistance observed in Escherichia coli isolates obtained from domestic- and wild-animal fecal samples, human septage, and surface water. Appl Environ Microbiol 71:1384–1404.

Singh KV, Murray BE. 2005. Differences in the Enterococcus faecalis population that influence susceptibility to quinupristin-dalfopristin and clindamycin. Antimicrob Agents Chemother 49:32–39.

Thurston-Enriquez JA, Gilley JE, Egglish B. 2005. Microbial quality of runoff from land application of cattle manure and swine slurry. J Water Health 3:157–171.

USDAs. 2006a. Farms, Land in Farms, and Livestock Operations: 2005 Summary: Washington, DC:U.S. Department of Agriculture. Available: http://usda.mannlib.cornell.edu/ussda/nass/MeatAnimPr/2005/MeatAnimPr-04-27-2006.pdf [accessed 13 September 2006].

USDAs. 2006b. Meat Animals Production, Disposition, and Income: 2005 Summary. Washington, DC:U.S. Department of Agriculture. Available: http://usda.mannlib.cornell.edu/ussda/nass/MeatAnimPr/2000s/2006/MeatAnimPr-04-27-2006.pdf [accessed 13 September 2006].

U.S. EPA. 2004. Wastes from Animal Feeding Operations: A Review of Science. Washington, DC:U.S. EPA. Available: www.epa.gov/nwcc/RecMan.pdf [accessed 13 September 2006].

U.S. EPA (U.S. Environmental Protection Agency). 2002. Drinking Water Contaminants. List of Drinking Water Contaminants & Their MCLs. EPA 816-F-02-013. Available: www.epa.gov/ drinkingwatercontaminants.html [accessed 13 September 2006].

U.S. EPA. 2003. Bacterial Water Quality Standards for Recreational Waters (Freshwater and Marine Waters). EPA-822-R-03-008. Washington, DC:U.S. Environmental Protection Agency. Available: http://www.epa.gov/waterscience/ beaches/local/state//Cape/I19ntgnted.html [accessed 12 September 2006].

U.S. Geological Survey. 2005. USGS Topographic Maps. Reston, VA:U.S. Geological Survey.

Wegener HC. 2003. Antibiotics in animal feed and their role in resistance development. Curr Opin Microbiol 6:439–445.