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# Airborne Multi-drug Resistant Bacteria Isolated from a Concentrated Swine Feeding Operation

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Short running head: Airborne Drug Resistant Bacteria in a Swine CAFO

Keywords: antibiotic resistance, multi-drug resistant bacteria, air, airborne bacteria, concentrated swine feeding operation, CAFO

Abbreviations:

Concentrated animal feeding operation (CAFO): Agricultural operation where large numbers of animals are kept and raised in confined conditions

Colony forming unit (CFU): Unit of measurement for viable bacteria

Minimal inhibitory concentration (MIC): The minimum antibiotic concentration that completely inhibits bacterial growth

Outline of manuscript section headers:

- 1) Abstract
- 2) Introduction
- 3) Materials and Methods
  - a) Study site
  - b) Collection of air samples
  - c) Bacterial isolation and speciation
  - d) Antimicrobial susceptibility testing
- 4) Results
  - a) Bacterial concentrations in air and bacterial identification
  - b) Antibiotic resistance
- 5) Discussion
- 6) Conclusions

**Abstract:**

The use of non-therapeutic levels of antibiotics in swine production can select for antibiotic resistance in commensal and pathogenic bacteria in swine. As a result, retail pork products, as well as surface and ground waters contaminated with swine waste, have been shown as sources of human exposure to antibiotic-resistant bacteria. However, it is unclear whether the air within swine operations also serves as a source of exposure to antibiotic-resistant bacterial pathogens. To investigate this issue, we sampled the air within a concentrated swine feeding operation with an all-glass impinger. Samples were analyzed using a method for the isolation of *Enterococcus*. One-hundred-thirty-seven presumptive *Enterococcus* isolates were identified to species level using standard biochemical tests and analyzed for resistance to erythromycin, clindamycin, virginiamycin, tetracycline and vancomycin using the agar dilution method. Thirty-four percent of the isolates were confirmed as *Enterococcus*, while 32% were identified as coagulase-negative staphylococci and 33% were identified as viridans group streptococci. Regardless of bacterial species, 98% of the isolates expressed high level resistance to at least two antibiotics commonly used in swine production. None of the isolates were resistant to vancomycin, an antibiotic that has never been approved for use in livestock in the U.S. In conclusion, high level multi-drug resistant *Enterococcus*, coagulase-negative staphylococci, and viridans group streptococci were detected in the air of a concentrated swine feeding operation. These findings suggest that the inhalation of air from these facilities may serve as an exposure pathway for the transfer of multi-drug resistant bacterial pathogens from swine to humans.

**Introduction:**

The development and persistence of multi-drug resistant bacteria poses increasing challenges to public health (IOM 1998). While the use of antibiotics in human medicine has influenced the emergence of antibiotic-resistant bacteria, the use of antibiotics in animal agriculture has markedly contributed to this critical problem as well (Cohen and Tauxe 1986; Gorbach 2001; IOM 1998; NRC 1999; van den Boogard and Stobberingh 1999). In animal agriculture, antibiotics are administered for therapeutic purposes to treat infections, prophylactic purposes in advance of observed symptoms and non-therapeutic purposes to promote growth and improve feed efficiency (Wegener, 2003). In general, antibiotics are administered at higher concentrations for therapeutic and prophylactic use and lower concentrations for non-therapeutic use (Wegener, 2003). It has been estimated that the non-therapeutic use of antimicrobials in livestock production comprises 60-80% of total antimicrobial production in the United States (Mellon et al. 2001). The swine industry alone uses an estimated 10.3 million pounds of antibiotics annually for non-therapeutic purposes. Among the antibiotics used are ampicillin, bacitracin, erythromycin, lincomycin, virginiamycin, and tetracycline (USFDA 2004), some of which are important in human clinical medicine. The use of antibiotics for non-therapeutic purposes such as growth promotion has been shown to select for resistance to high concentrations of antibiotics in both pathogenic and commensal bacteria in swine (Aarestrup et al. 2000a, 2000b; Bager et al. 1997; Jensen et al. 2002; Wegener et al. 1999). For this reason, attention has been given to retail pork products as a source of human exposure to antibiotic-resistant bacteria (Donabedian et al 2003; Gambarotto et al. 2001; Hayes et al. 2003; Sorensen et al. 2001; White et al. 2001). Yet the ingestion of pork products is not the only pathway of exposure for the transfer of resistant

organisms from swine to humans. Environmental pathways of exposure may be equally important.

Along with the pork products, over 110 million tons of swine waste—containing antibiotic-resistant bacteria—is produced at swine concentrated animal feeding operations (CAFOs) in the U.S. each year (ED 1997). The practice of storing this waste in pits and open air lagoons and subsequently applying the waste to land can lead to the contamination of soils and nearby surface and ground waters. Several studies have reported the appearance of antibiotic residues and antibiotic-resistant bacteria in surface and ground waters proximal to swine CAFOs (Campagnolo et al. 2002; Chee-Sanford et al. 2001). Campagnolo et al. suggest that swine waste may be a source of antimicrobial drugs in surface and ground waters near swine facilities, (Campagnolo et al. 2002) and Chee-Sanford et al. found that groundwater can be impacted by swine waste and serve as a potential source of exposure to antibiotic-resistance genes (Chee-Sanford et al. 2001).

However, few studies have examined the air within swine CAFOs as an additional environmental exposure source for antibiotic-resistant bacterial pathogens. It has been well-documented that the air within swine CAFOs is highly contaminated with bacteria, yeasts and molds. Mean total bacterial concentrations can range from  $10^4$  colony-forming units/m<sup>3</sup> (CFU/m<sup>3</sup>) to  $10^7$  CFU/m<sup>3</sup> (Clark et al. 1983; Cormier et al. 1990; Crook et al. 1991; Predicala et al. 2002). Specific bacteria detected in the air of swine CAFOs have included the following potential human pathogens: *Enterococcus*, *Staphylococcus*, *Pseudomonas*, *Bacillus*, *Listeria*, and *Escherichia coli* (Cormier et al. 1990; Crook et al. 1991; Predicala et al. 2002). Yet, to date, these airborne pathogens have

not been assessed for resistance to antibiotics that are commonly used in both swine production and clinical medicine. Hamscher et al. assessed the presence of antibiotics in dust samples collected at a swine production facility over two decades (Hamscher et al. 2003). Several different antibiotics, including tetracycline, tylosin (an analog to erythromycin) and chloramphenicol, could be detected in 90% of the dust samples tested (Hamscher et al. 2003). In abstract form within conference proceedings, Zahn et al. reported on the presence of tylosin and tylosin-resistant bacteria in the air released from three mechanically-ventilated swine CAFOs (Zahn et al. 2001). Their study indicated that tylosin-resistant bacteria, primarily *Corynebacterium*, accounted for 80% of total culturable bacteria detected. These results provided the first evidence of airborne antibiotic-resistant bacteria in swine CAFOs.

The goal of this study was to test air samples collected within a swine CAFO for the presence of antibiotic-resistant enterococci, gram-positive, catalase-negative cocci that are not only members of the normal intestinal flora of humans and animals but also capable of causing a variety of human and animal infections (Anonymous 2001). Resistance to erythromycin, clindamycin, tetracycline, and virginiamycin (an analog to quinupristin/ dalfopristin, which is used to treat vancomycin-resistant *Enterococcus faecium* infections in humans (Johnson and Livermore 1999)) was investigated. These drugs (or their analogs) have been approved for use in swine production for growth promotion, feed efficiency and therapeutic purposes. Resistance to vancomycin also was tested. Vancomycin, an analog to avoparcin, which has been used extensively in animal agriculture in Europe, has never been approved for use in livestock in the United States.

## **Materials and Methods:**

### *Study site*

The study site is a swine finishing CAFO located in the Mid-Atlantic United States. The CAFO consists of two tunnel-ventilated swine houses built atop 12-ft deep concrete pits where swine waste is stored prior to periodic siphoning into a transport truck for off-farm disposal by land application. Each house has the capacity to hold 2,500 hogs; however, during the sampling period approximately 1,500 hogs were being housed in each building. Air sampling at the swine facility was conducted on December 9, 2003 and January 5, 2004.

### *Collection of air samples*

Air samples were collected at a calibrated flow rate of 12.5 liters per minute using an all-glass impinger (AGI-30, Ace Glass, Vineland, NJ) designed to collect respirable particles, including bioaerosols, with an aerodynamic diameter less than 5  $\mu\text{m}$  (impingers were autoclaved and filled with 20 ml of phosphate buffered saline prior to sampling). On the first day, sampling was conducted over a 30 min period. On the second day, sampling was conducted for 60 min in order to increase yield. For the longer sampling period, the impinger solution was replenished with distilled deionized H<sub>2</sub>O to maintain the sampler collection efficiency, (Lin et al. 1997) and avoid increasing liquid salinity. All sampling equipment was placed on top of a table (1.5 m from the ground) within an empty swine stall located within the facility approximately 30 meters from the south wall of the swine facility where air exits through ventilation fans. At the time of sampling, four of eight 32-inch ventilation fans were in operation to maintain a farm-operator-designated target temperature of 21° C within the facility. Temperature and relative humidity

were monitored throughout the sampling periods and were 22° C +/- 1° C and 76% +/- 4% respectively. Impingers were stored and transported back to the laboratory at 4°C.

### *Bacterial isolation and speciation*

Approximately 3 hours after the last air sample was collected, impinger liquid samples were analyzed in the laboratory. Since no standard method exists regarding the isolation of *Enterococcus* from air, the standard methods utilized for the isolation of *Enterococcus* from recreational water were modified to accommodate the air samples (USEPA 2000). All broths and agars were obtained from Becton Dickinson, Sparks, MD. Three 10-fold dilutions (using phosphate buffered saline as the diluent) of the impinger samples were plated (100 µl/plate) in duplicate on mE agar. Negative control plates were made by plating 100 µl of both the replenishing fluid that was transported to the site and the dilution liquid. All plates were incubated for 48 hours at 41.5° C under aerobic conditions. All resulting colonies were counted and counts from dilution plates containing 30 to 300 CFUs were used in back-calculations to determine the concentration of isolated bacteria per m<sup>3</sup> of air within the swine CAFO. Colonies from sample dilution plates that ranged from pink to red in color (indicative of *Enterococcus* colonies) were streaked onto Enterococcosel agar and incubated for 24 hours at 41.5°C under aerobic conditions. CFUs characteristic of *Enterococcus* that formed a black precipitate on the Enterococcosel agar plates were considered presumptive *Enterococcus* (USEPA, 2000). Presumptive *Enterococcus* isolates were archived in a 20% glycerol, tryptic soy broth solution at -80° C for subsequent speciation and antimicrobial susceptibility testing.

All presumptive *Enterococcus* isolates, as well as the quality control strains *Enterococcus faecium* 19434 and *Enterococcus faecalis* 29212 (ATCC, Manassas, VA), were streaked from -80° C archived stocks onto both tryptic soy agar and tryptic soy agar No.2 with 5% defibrinated sheep blood (Quad Five, Ryegate, MT) and incubated for 24 hours at 37° C. All of the media formulations and test interpretations used are described in the *Manual of Clinical Microbiology*, 8<sup>th</sup> Edition (Murray et al. 2003). Gram stains were prepared on all isolates to verify the presence of gram-positive cocci. Each isolate was tested for the production of catalase in the presence of 3% hydrogen peroxide. Catalase-positive isolates were identified as *Staphylococcus spp.* (except for 1 isolate which was further tested for oxidase activity and identified as *Micrococcus luteus*). Each *Staphylococcus* isolate was inoculated onto 0.5 ml rabbit plasma (Becton Dickinson BBL, Sparks, MD) to test for the production of coagulase. Catalase-negative isolates were differentiated further by pyrrolidonyl-arylamidase activity using REMEL's PYR Kit (Remel, Lenexa, KS). The following biochemical tests were performed on the isolates displaying pyrrolidonyl-arylamidase activity: mannitol, arabinose, sorbitol, raffinose, lactose and sucrose carbohydrate fermentation tests; arginine deamination; acidification of methyl- $\alpha$ -D-glucopyranoside; pyruvate utilization; and isolate pigmentation.

#### *Antimicrobial susceptibility testing*

Antimicrobial susceptibility testing was conducted using the minimal inhibitory concentration (MIC) agar dilution method (NCCLS 2002). *Enterococcus faecalis* 29212 was used as the quality control reference strain. Susceptibility to erythromycin, clindamycin, virginiamycin (streptogramin A and B combination), tetracycline and vancomycin was tested. Erythromycin,

clindamycin, tetracycline and vancomycin were obtained from Sigma, St. Louis, MO.

Virginiamycin was obtained from Research Products International Corp., Mt. Prospect, IL.

Concentrations of antibiotics tested ranged from 0.5 µg/ml to 256 µg/ml for erythromycin and tetracycline, 0.03 µg/ml to 128 µg/ml for clindamycin, 0.03 µg/ml to 32 µg/ml for virginiamycin, and 0.03 µg/ml to 64 µg/ml for vancomycin.

In preparation for the agar dilution tests, the air sample isolates, as well as the MIC reference strain *Enterococcus faecalis* 29212, were streaked from -80° C archived stocks onto tryptic soy agar No. 2 with 5% defibrinated sheep blood (QuadFive, Ryegate, MT) and incubated for 24 hours at 37° C. After 24 hours, each isolate was suspended in 3 ml Mueller-Hinton broth with a sterile cotton swab and adjusted to a 0.5 McFarland standard using a Vitek colorimeter (Hach, Loveland, CO). Two-hundred µl of each suspension were transferred to a well within a Cathra replicator plate (Oxoid Inc., Ogdensburg, NY) and replicated with 1 mm pins in accordance with National Committee for Clinical Laboratory Standards (NCCLS) guidelines onto Mueller-Hinton agar plates that were previously prepared with the appropriate concentrations of antibiotics (NCCLS 2002). Plates were incubated for 24 hours at 37° C under aerobic conditions. After 24 hours, the plates were read manually and MICs were determined. Specifically, the MIC was recorded as the minimum antibiotic concentration that completely inhibited bacterial growth. According to the MIC, isolates were categorized as susceptible, intermediate or resistant to each antibiotic using the following MIC breakpoints established by the NCCLS for *Enterococcus*: erythromycin, susceptible  $\leq 0.5$  µg/ml, intermediate 1-4 µg/ml, and resistant  $\geq 8$  µg/ml; clindamycin, susceptible  $\leq 0.5$  µg/ml, intermediate 1-2 µg/ml, and resistant  $\geq 4$  µg/ml; virginiamycin, susceptible  $\leq 1$  µg/ml, intermediate 2 µg/ml, and resistant  $\geq 4$  µg/ml;

tetracycline, susceptible  $\leq 4 \mu\text{g/ml}$ , intermediate  $8 \mu\text{g/ml}$ , and resistant  $\geq 16 \mu\text{g/ml}$ ; and vancomycin, susceptible  $\leq 4 \mu\text{g/ml}$ , intermediate  $8\text{-}16 \mu\text{g/ml}$ , and resistant  $\geq 32 \mu\text{g/ml}$  (NCCLS 2002).

## **Results:**

### *Bacterial concentrations in air and bacterial identification*

The mean concentration of presumptive *Enterococcus* present in the air of the swine CAFO on both December 9, 2003 and January 5, 2004 was  $4 \times 10^4 \text{ CFU/m}^3$ . After bacterial speciation was completed on 137 presumptive *Enterococcus* isolates, only 47 out of 137 isolates (34%) were confirmed to be *Enterococcus* (Table 1). Forty four isolates (32%) were identified as staphylococci, 45 isolates (33%) were viridans group streptococci, and 1 isolate was identified as *Micrococcus luteus* (Table 1).

### *Antibiotic resistance*

Ninety-eight percent (121 out of 124) of the bacterial isolates that grew successfully during the antimicrobial susceptibility tests were resistant to high levels of at least two antibiotics commonly used in swine production (erythromycin, clindamycin, virginiamycin or tetracycline), and 93% of the isolates (115 out of 124) were resistant to three antibiotics commonly used in swine production. Individually, 98% of the isolates were resistant to erythromycin, 94% were resistant to clindamycin, 90% were resistant to tetracycline and 37% were resistant to virginiamycin. None of the isolates displayed resistance to vancomycin. Since none of the *E. avium*, *E. pseudoavium*, or *E. raffinosus* isolates (all belonging to the *Enterococcus* physiological Group I) grew successfully on the control or antibiotic-amended MIC plates after being

suspended as 0.5 McFarland standard solutions, MIC data for these isolates were not determined. MIC distributions among all other isolates were similar for erythromycin, clindamycin, tetracycline and vancomycin, regardless of bacterial genus or species (Table 2 and Table 3). For instance, across all organisms, the majority of isolates (96%) had MICs > 256 µg/ml for erythromycin (Table 2 and Table 3). In contrast, resistance to virginiamycin was more prevalent among coagulase-negative staphylococci versus *Enterococcus* or *Streptococcus* isolates (Table 2 and Table 3). Phenotypes of antibiotic resistance among the bacterial isolates appear in Table 4.

### **Discussion:**

In this study, multi-drug resistant *Enterococcus*, coagulase-negative staphylococci, and viridans group streptococci were isolated from the air of a swine CAFO. Ninety-eight percent of the isolates were resistant to at least two of the following antibiotics: erythromycin, clindamycin, virginiamycin and tetracycline, all of which are approved for use in swine production for growth promotion. In contrast, none of the isolates were resistant to vancomycin, which has never been approved for use in swine production in the U.S. These results support the findings of previous reports that non-therapeutic use of antibiotics results in the presence of antibiotic-resistant bacteria in swine (Aarestrup et al. 2000a, 2000b; Bager et al. 1997; Jensen et al. 2002; Wegener et al. 1999). In addition, these results provide evidence that in the absence of non-therapeutic antibiotic use—vancomycin in this case—no resistance is detected among bacteria present in the swine environment.

Furthermore, these findings suggest that, in addition to the ingestion of retail pork products (Gambarotto et al. 2001; Hayes et al. 2003; Sorensen et al. 2001; White et al. 2001) and surface

and ground waters in the vicinity of swine CAFOs, (Campagnolo et al. 2002; Chee-Sanford et al. 2001) the inhalation of air within swine operations may serve as an additional exposure pathway for the transfer of multi-drug resistant bacteria from swine to humans. These data are especially relevant to the health of swine CAFO workers, their direct contacts in the community, and possibly nearby neighbors of swine CAFOs.

The types of bacteria detected within the air of the swine facility investigated in this study are associated with a variety of human infections. *Enterococcus*, particularly some of the species isolated in this study including *E. faecalis* and *E. faecium*, has emerged as one of the leading causes of nosocomial bacteremias, urinary tract infections, and wound infections in the U.S. (Anonymous 2001). Similarly, coagulase-negative staphylococci are the third most common causes of nosocomial infections and the most common causes of nosocomial bacteremias. The presence of multi-drug resistant *Enterococcus* and coagulase-negative staphylococci in patients significantly limits the treatment options available for these life-threatening infections. Although viridans group streptococci comprise the normal flora of the human respiratory tract, they also have been implicated as the cause of infective endocarditis and life-threatening septicemias in neutropenic patients. In addition, viridans group streptococci have been implicated as reservoirs of erythromycin-resistance genes, possibly capable of transferring resistance determinants to more pathogenic species including *Streptococcus pneumoniae* and *Streptococcus pyogenes* (Bryskier 2002).

Of particular concern to the health of individuals with direct or indirect contact with swine environments is the finding of virginiamycin-resistant gram-positive bacteria in the air of the

swine CAFO. Virginiamycin, a streptogramin A and B combination, which has been used extensively as a growth promoter in swine, is an analog to quinupristin-dalfopristin, an injectable streptogramin A and B combination that is often the drug of last resort for multi-drug resistant gram-positive infections characterized by methicillin-resistant *Staphylococcus aureus* and glycopeptide-resistant *Enterococcus faecium* and coagulase-negative staphylococci (Johnson and Livermore 1999). Bacteria expressing resistance to virginiamycin are cross-resistant to quinupristin-dalfopristin, and a previous study has suggested that the transfer of streptogramin-resistant *Enterococcus* can occur between animals and humans in the livestock environment (Jensen et al. 1998). Thus, the inhalation of virginiamycin-resistant gram-positive bacteria in the swine environment could contribute to the appearance of quinupristin-dalfopristin-resistant gram-positive infections in humans, leaving little to no treatment options for the affected individual.

The finding of airborne clindamycin-resistant gram-positive bacteria in this study also is a potential concern to public health. Clindamycin is indicated for the treatment of human staphylococcal and streptococcal pneumonia (among other aerobic and anaerobic infections). Specifically, clindamycin has been utilized for the treatment of community-acquired methicillin-resistant *S. aureus* (Marcinak and Arthur 2003). Clindamycin also has been shown to be significantly more potent than penicillin in inhibiting both invasive and non-invasive group A streptococci such as *S. pyogenes* (Mascini et al. 2001). The findings of airborne clindamycin-resistant coagulase-negative staphylococci and viridans group streptococci in the swine environment raise the question as to whether these organisms could serve as reservoirs of clindamycin-resistant genes (as well as reservoirs of erythromycin resistant genes (Bryskier,

2002)), passing on clindamycin resistance determinants to more pathogenic species as described above.

Furthermore, exposure to virginiamycin-, erythromycin-, clindamycin- and tetracycline-resistant *Enterococcus*, coagulase-negative staphylococci, and viridans group streptococci through the inhalation of contaminated air could lead to the colonization of these multi-drug resistant organisms in both the nasal passages (Aubry-Damon 2004) and the lungs of swine CAFO workers, potentially making the workers themselves reservoirs of antibiotic-resistant organisms. Co-exposures to other aerosols and gases in the swine environment such as organic dusts, molds and ammonia have been shown to induce symptoms associated with chronic bronchitis including a persistent cough characterized by expectoration (Mackiewicz 1998). The presence of this type of cough can increase the potential for secondary spread of antibiotic-resistant organisms into the community, where additional individuals could serve as reservoirs of multi-drug resistant bacteria.

Moreover, the tunnel-ventilated design of swine CAFOs, which moves air outside of the facilities at a high flow rate, could create a situation where neighbors living downwind of the ventilation fans also could be directly exposed to airborne multi-drug resistant bacteria. An epidemiological study by Wing and Wolf (2000) indicated that people who live in the vicinity of swine CAFOs experience elevated rates of headaches, runny noses, sore throats, excessive coughing and diarrhea compared to people living in communities that are not situated near livestock operations (Wing and Wolf 2000). The findings of airborne multi-drug resistant bacteria in a swine CAFO in our study raise the question as to whether airborne bacteria also

could travel beyond the confines of the swine CAFO on ventilation fan air currents, directly contacting nearby neighbors and potentially contributing to health effects such as those observed in the Wing and Wolf study. Since populations living in areas where swine CAFOs are built already may experience higher rates of certain diseases due to lack of access to appropriate healthcare (Weber et al. 1989), investigating airborne exposures to multi-drug resistant bacteria among these at-risk populations is an important area for future research.

In addition to potential airborne exposures occurring among individuals living near swine CAFOs, the results of this study could have broader public health implications. Specifically, one may question whether airborne exposures to multi-drug resistant bacteria could be occurring and contributing to health problems around other environmental sources of animal or human waste, including land application areas for animal waste and human sludge, and human wastewater treatment facilities. Endotoxins, exotoxins and other chemical components in dusts associated with animal waste and human sludge have been linked to hypersensitivity reactions among individuals living near land application areas (Lewis and Gattie 2000). These reactions have been shown to result in increased susceptibility to serious respiratory infections, including those caused by *S. aureus* (Lewis and Gattie 2000). Thus, the presence of high concentrations of multi-drug resistant staphylococci and other bacterial pathogens amidst endotoxin-containing dust from animal and human waste could pose unique health concerns to people living near land application areas.

## **Conclusions:**

In summation, the findings of this study suggest that the inhalation of air from swine CAFOs may serve as an additional environmental exposure pathway for the transfer of multi-drug resistant bacterial pathogens from swine to humans. Given the growing interest in reservoirs of antibiotic resistance genes associated with large-scale livestock operations, (Nandi et al. 2004) the findings of this investigation emphasize the importance of studying multiple genera of bacteria in different environmental media as sources of human exposure to antibiotic resistance genes.

## Reference List

- Aarestrup FM, Agerso Y, Gerner-Smidt P, Madsen M, Jensen LB. 2000a. Comparison of antimicrobial resistance phenotypes and resistance genes in *Enterococcus faecalis* and *Enterococcus faecium* from humans in the community, broilers, and pigs in Denmark. *Diagn Microbiol Infect Dis* 37:127-137.
- Aarestrup FM, Kruse H, Tast E, Hammerum AM, Jensen LB. 2000b. 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.
- [Anonymous.] 2001. National Nosocomial Infections Surveillance (NNIS) System Report, Data Summary from January 1992-June 2001. *Am J Infect Control* 29:404-421.
- Aubry-Damon H. 2004. Antimicrobial resistance in commensal flora of pig farmers. *Emerg Infect Dis* 10:873-879.
- Bager F, 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.
- Bryskier A. 2002. Viridans group streptococci: a reservoir of resistant bacteria in oral cavities. *Clin Microbiol Infect* 8:65-69.

Campagnolo ER, Johnson KR, Karpati A, Rubin CS, Kolpin DW, Meyer 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.

Chee-Sanford JC, Aminov RI, Krapac IJ, Garrigues-Jeanjean N, Mackie RI. 2001. Occurrence and diversity of tetracycline resistance genes in lagoons and groundwater underlying two swine production facilities. *Appl Environ Microbiol* 67:1494-1502.

Clark S, Rylander R, Larsson L. 1983. Airborne bacteria, endotoxin and fungi in dust in poultry and swine confinement buildings. *Am Ind Hyg Assoc J* 44:537-541.

Cohen ML, Tauxe RV. 1986. Drug-resistant *Salmonella* in the United States: an epidemiologic perspective. *Science* 234:964-969.

Cormier Y, Tremblay G, Meriaux A, Brochu G, Lavoie J. 1990. Airborne microbial contents in two types of swine confinement buildings in Quebec. *Am Ind Hyg Assoc J* 51:304-309.

Crook B, Robertson JF, Glass SA, Botheroyd EM, Lacey J, Topping MD. 1991. Airborne dust, ammonia, microorganisms, and antigens in pig confinement houses and the respiratory health of exposed farm workers. *Am Ind Hyg Assoc J* 52:271-279.

Donabedian SM, Thal LA, Hershberger E, Perri MB, Chow JW, Bartlett P, et al. 2003.

Molecular characterization of gentamicin-resistant *Enterococci* in the United States: evidence of spread from animals to humans through food. *J Clin Microbiol* 41:1109-13.

ED (Environmental Defense). 1997. Animal waste summary. New York, NY:Environmental Defense. Available: <http://www.hogwatch.org/maps/index> [accessed 15 June 2004].

Gambarotto K, Ploy MC, Dupron F, Giangiobbe M, Denis F. 2001. Occurrence of vancomycin-resistant enterococci in pork and poultry products from a cattle-rearing area of France. *J Clin Microbiol* 39:2354-2355.

Gorbach SL. 2001. Antimicrobial use in animal feed--time to stop. *N Engl J Med* 345:1202-1203.

Hamscher G, Pawelzick HT, Sczesny S, Nau H, Hartung J. 2003. Antibiotics in dust originating from a pig-fattening farm: a new source of health hazard for farmers? *Environ Health Perspect* 111:1590-1594.

Hayes JR, English LL, Carter PJ, Proescholdt T, Lee KY, Wagner DD, et al. 2003. Prevalence and antimicrobial resistance of enterococcus species isolated from retail meats. *Appl Environ Microbiol* 69:7153-7160.

IOM. 1998. Antimicrobial Resistance: Issues and Options, Workshop Report, Forum on Emerging Infections. Washington, D.C.:National Academy Press.

Jensen LB, Hammerum AM, Aarestrup FM, van den Bogaard AE, Stobberingh EE. 1998. Occurrence of satA and vgb genes in streptogramin-resistant *Enterococcus faecium* isolates of animal and human origins in the Netherlands. *Antimicrob Agents Chemother* 42:3330-3331.

Jensen LB, Hammerum AM, Bager F, and Aarestrup FM. 2002. Streptogramin resistance among *Enterococcus faecium* isolated from production animals in Denmark in 1997. *Microb Drug Resist* 8:369-374.

Johnson AP, Livermore DM. 1999. Quinupristin/dalfopristin, a new addition to the antimicrobial arsenal. *Lancet* 354:2012-2013.

Lewis DL, Gattie DK. 2002. Pathogen risks from applying sewage sludge to land. *Environ Sci Technol* 36:286A-293A.

Lin X, Willeke K, Ulevicius V, Grinshpun S. 1997. Effect of sampling time on the collection efficiency of all-glass impingers. *Am Ind Hyg Assoc J* 58:480-488.

Mackiewicz B. 1998. Study on exposure of pig farm workers to bioaerosols, immunologic reactivity and health effects. *Ann Agric Environ Med* 5:169-175.

Marcinak JF, Frank AL. 2003. Treatment of community-acquired methicillin-resistant *Staphylococcus aureus* in children. *Curr Opin Infect Dis* 16:265-269.

Mascini EM, Janszea M, Schoulsb LM, Verhoefa J, Van Dijk H. 2001. Penicillin and clindamycin differentially inhibit the production of pyrogenic exotoxins A and B by group A streptococci. *Int J Antimicrob Agents* 18:395-398.

Mellon M, Benbrook C, Benbrook KL. 2001. *Hogging It: Estimates of Antimicrobial Abuse in Livestock*. Cambridge, MA:Union of Concerned Scientists Publications.

Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Tenover FC, Tenover FC. 2003. *Manual of Clinical Microbiology*. 8<sup>th</sup> ed. Washington, D.C.:American Society for Microbiology Press.

Nandi S, Maurer JJ, Hofacre C, Summers AO. 2004. Gram-positive bacteria are a major reservoir of Class 1 antibiotic resistance integrons in poultry litter. *Proc Natl Acad Sci U.S.A* 101:7118-7122.

NCCLS. 2002. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolates from animals; Approved Standard--Second edition. M31-A2 [ISBN 1-56238-461-9]. Wayne, PA:NCCLS.

NRC. 1999. The use of drugs in food animals: Benefits and risks. Washington, D.C.:National Academy Press.

Predicala BZ, Urban JE, Maghirang RG, Jerez SB, Goodband RD. 2002. Assessment of bioaerosols in swine barns by filtration and impaction. *Curr Microbiol* 44:136-140.

Sorensen TL, Blom M, Monnet DL, Frimodt-Moller N, Poulsen RL, Espersen F. 2001. Transient intestinal carriage after ingestion of antibiotic-resistant *Enterococcus faecium* from chicken and pork. *N Engl J Med* 345:1161-1166.

USEPA. 2000. Improved enumeration methods for the recreational water quality indicators: Enterococci, and *Escherichia coli*. EPA/821/R-97/004. Washington, D.C: U.S. Environmental Protection Agency.

USFDA. 2004. FDA approved animal drug products. Blacksburg, VA:Drug Information Laboratory, Virginia/Maryland Regional College of Veterinary Medicine.

van den Boogard AE, Stobberingh, EE. 1999. Antibiotic usage in animals: impact on bacterial resistance and public health. *Drugs* 58:589-607.

Weber D, Rutala W, Samsa G, Sarubbi F, King L. 1989. Epidemiology of tuberculosis in North Carolina, 1966 to 1986: analysis of demographic features, geographic variation, AIDS, migrant workers, and site of infection. *South Med J* 92:1204-1214.

Wegener HC, Aarestrup FM, Jensen LB, Hammerum AM, Bager F. 1999. Use of antimicrobial growth promoters in food animals and *Enterococcus faecium* resistance to therapeutic antimicrobial drugs in Europe. *Emerg Infect Dis* 5:329-335.

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

Wing S and Wolf S. 2000. Intensive livestock operations, health, and quality of life among Eastern North Carolina residents. *Environ Health Perspect* 108:233-238.

White DG, Zhao S, Sudler R, Ayers S, Friedman S, Chen S, et al. 2001. The isolation of antibiotic-resistant salmonella from retail ground meats. *N Engl J Med* 345:1147-1154.

Zahn JA, Anhalt J, Boyd E. 2001. Evidence for transfer of tylosin and tylosin-resistant bacteria in air from swine production facilities using sub-therapeutic concentrations of tylosin in feed. *J Anim Sci* 79:189.

**Table 1:** Airborne bacteria isolated from a concentrated swine feeding operation using methods for the isolation of *Enterococcus spp.*

<b>Bacteria</b>	<b>Number of Isolates (%)</b>
<i>Enterococcus</i>	47 (34)
<i>E. avium</i>	5 (4)
<i>E. dispar</i>	4 (3)
<i>E. durans</i>	2 (1)
<i>E. faecalis</i>	6 (4)
<i>E. faecium</i>	1 (<1)
<i>E. hirae</i>	14 (10)
<i>E. mundtii</i>	1 (<1)
<i>E. pseudoavium</i>	2 (1)
<i>E. raffinosus</i>	1 (<1)
Other	11 (8)
<i>Staphylococcus</i>	44 (32)
<i>S. aureus</i>	1 (<1)
Coagulase-negative staphylococci	43 (31)
<i>Streptococcus</i>	
Viridans group streptococci	45 (33)
<i>Micrococcus luteus</i>	1 (<1)
Total	137 (100)

**Table 2:** Minimal inhibitory concentration (MIC) distributions for five antibiotics observed in airborne *Enterococcus* collected from a concentrated swine feeding operation

Bacteria	Antibiotic	Number of bacterial isolates with the following MICs ( $\mu\text{g/ml}$ ):										%S <sup>a</sup>	%I <sup>b</sup>	%R <sup>c</sup>	
		$\leq 0.5$	1	2	4	8	16	32	64	128	256				>256
<i>Enterococcus</i> (n=38)	Erythromycin			1								37	0	3	97
	Clindamycin	1				1	1	1	3	8	23 <sup>d</sup>		3	0	97
	Virginiamycin	19	5	5	9								63	13	24
	Tetracycline				1	2	7	6	17	5			3	5	92
	Vancomycin	38											100	0	0
<i>E. dispar</i> (n=4)	Erythromycin											4	0	0	100
	Clindamycin							1	1	2 <sup>d</sup>			0	0	100
	Virginiamycin	4											100	0	0
	Tetracycline							3	1				0	0	100
	Vancomycin	4											100	0	0
<i>E. durans</i> (n=2)	Erythromycin											2	0	0	100
	Clindamycin								1	1 <sup>d</sup>			0	0	100
	Virginiamycin			1	1								0	50	50
	Tetracycline				1	1							50	50	0
	Vancomycin	2											100	0	0
<i>E. faecalis</i> (n=6)	Erythromycin	1										5	17	0	83
	Clindamycin	1				1				2	2 <sup>d</sup>		17	0	83
	Virginiamycin	2	3		1								83	0	17
	Tetracycline						2	1	2	1			0	0	100
	Vancomycin	6											100	0	0
<i>E. faecium</i> (n=1)	Erythromycin											1	0	0	100
	Clindamycin								1				0	0	100
	Virginiamycin				1								0	0	100
	Tetracycline						1						0	0	100
	Vancomycin	1											100	0	0
<i>E. hirae</i> (n=14)	Erythromycin											14	0	0	100
	Clindamycin								2	12 <sup>d</sup>			0	0	100
	Virginiamycin	8		2	4								57	14	29
	Tetracycline					1	2	1	8	2			0	7	93
	Vancomycin	14											100	0	0
Other (n=11)	Erythromycin											11	0	0	100
	Clindamycin						1	1	2	1	6 <sup>d</sup>		0	0	100
	Virginiamycin	5	2	2	2								64	18	18
	Tetracycline						2	4	4	1			0	0	100
	Vancomycin	11											100	0	0

<sup>a</sup> % Susceptible

<sup>b</sup> % Intermediate

<sup>c</sup> % Resistant

<sup>d</sup> MIC is > 128  $\mu\text{g/ml}$

**Table 3:** Minimal inhibitory concentration (MIC) distributions for five antibiotics observed in airborne *Staphylococcus* and *Streptococcus* collected from a concentrated swine feeding operation

Bacteria	Antibiotic	Number of bacterial isolates with the following MICs ( $\mu\text{g/ml}$ ):										%S <sup>a</sup>	%I <sup>b</sup>	%R <sup>c</sup>		
		$\leq 0.5$	1	2	4	8	16	32	64	128	256				>256	
<i>Staphylococcus</i> (n=43) <sup>d</sup>																
<i>S. aureus</i> (n=1)	Erythromycin											1	0	0	100	
	Clindamycin											1 <sup>e</sup>	0	0	100	
	Virginiamycin			1									0	100	0	
	Tetracycline								1				0	0	100	
	Vancomycin	1											100	0	0	
Coag.-negative staphylococci (n=42)	Erythromycin												42	0	0	100
	Clindamycin		1								2	39 <sup>e</sup>	0	2	98	
	Virginiamycin	4	2	2	21	13							14	5	81	
	Tetracycline		2		1		1	5	12	13	7	1	7	0	93	
	Vancomycin	7	3	30	2								100	0	0	
<i>Streptococcus</i> (n=43) <sup>f</sup>																
Viridans group streptococci	Erythromycin			1			1	1					40	0	0	100
	Clindamycin	2						2	2	9			28	5	0	95
	Virginiamycin	29	7	4	3								84	9	7	
	Tetracycline	1					8	17	10	7			2	0	98	
	Vancomycin	43											100	0	0	

<sup>a</sup> % Susceptible

<sup>b</sup> % Intermediate

<sup>c</sup> % Resistant

<sup>d</sup> Analyzed using the breakpoints for *Enterococcus*

<sup>e</sup> MIC is > 128  $\mu\text{g/ml}$

<sup>f</sup> Analyzed using the following breakpoints: erythromycin, susceptible  $\leq 0.25$   $\mu\text{g/ml}$ , intermediate 0.5  $\mu\text{g/ml}$ , and resistant  $\geq 1.0$   $\mu\text{g/ml}$ ; clindamycin, susceptible  $\leq 0.5$   $\mu\text{g/ml}$ , intermediate 1-2  $\mu\text{g/ml}$ , and resistant  $\geq 4$   $\mu\text{g/ml}$ ; virginiamycin, susceptible  $\leq 1$   $\mu\text{g/ml}$ , intermediate 2  $\mu\text{g/ml}$ , and resistant  $\geq 4$   $\mu\text{g/ml}$ ; tetracycline, susceptible  $\leq 2$   $\mu\text{g/ml}$ , intermediate 4  $\mu\text{g/ml}$ , and resistant  $\geq 8$   $\mu\text{g/ml}$ ; vancomycin, susceptible  $\leq 1$   $\mu\text{g/ml}$ , intermediate not available, resistant not available (NCCLS, 2002).

**Table 4:** Phenotypes of antibiotic resistance among airborne bacteria collected from a concentrated swine feeding operation

<b>Bacteria</b>	<b>Antibiotic Resistance Pattern<sup>a</sup></b>	<b>Number of Isolates (%)</b>
<i>E. dispar</i> (n=4)	Ery, Clin, Tet	4(100)
<i>E. durans</i> (n=2)	Ery, Clin	1(50)
	Ery, Clin, Virg	1(50)
<i>E. faecalis</i> (n=6)	Tet	1(17)
	Ery, Clin, Tet	4(66)
	Ery, Clin, Tet, Virg	1(17)
<i>E. faecium</i> (n=1)	Ery, Clin, Tet, Virg	1(100)
<i>E. hirae</i> (n=14)	Ery, Clin	1(7)
	Ery, Clin, Tet	9(64)
	Ery, Clin, Tet, Virg	4(29)
Other <i>Enterococcus</i> (n=11)	Ery, Clin, Tet	9(82)
	Ery, Clin, Tet, Virg	2(18)
<i>S. aureus</i> (n=1)	Ery, Clin, Tet	1(100)
Coag.-negative staphylococci (n=42)	Ery, Tet	1(2)
	Ery, Clin, Tet	8(19)
	Ery, Clin, Virg	6(14)
	Ery, Virg, Tet	1(2)
	Ery, Clin, Tet, Virg	26(62)
Viridans group streptococci (n=43)	Tet	2(5)
	Ery, Clin	1(2)
	Ery, Tet	2(5)
	Ery, Clin, Tet	35(81)
	Ery, Clin, Tet, Virg	3(7)

<sup>a</sup> Ery=erythromycin, Tet=tetracycline, Clin=clindamycin, and Virg=virginiamycin