EVALUATION OF BIOAEROSOLS EMITTED FROM SWINE CONFINEMENT OPERATIONS

Shawn G. Gibbs[1] and Pasquale V. Scarpino[2]

[1]The University of Texas Health Science Center
School of Public Health, El Paso Regional Campus
El Paso TX, 79902

[2]Department of Civil and Environmental Engineering
University of Cincinnati
Cincinnati, Ohio 45221-0071

ABSTRACT

The creation of antibiotic resistant bacteria is a global problem that the United States is just now starting to address. Hospital costs to treat antibiotic resistance are estimated to be hundreds of millions of dollars a year, and jeopardize the health of the human or animal that is infected. The inhalation of large amounts of airborne microorganisms is also a problem when dealing with those that work with or live in proximity to animal confinement units. It has been shown that the inhalation of large numbers of microorganisms creates a variety of adverse human health effects.

The air samples in this article were taken from upwind, inside, and downwind of two swine confinement units using Andersen Two-Stage Viable Microbial Particle Sizing Sampler Instruments. These samples were then used to calculate the CFUs/m^3 for both bacteria and fungi, which were both, found to be in high levels downwind and inside of the swine production facilities. The organisms were then identified to assess their pathogenicity to human health. The organisms isolated from each site were then tested for antibiotic resistance using the Kirby-Bauer method of disc diffusion. Resistance was discovered inside and downwind of the swine confinement facilities,

indicating that the resistant organisms were being produced in and released from these facilities. Resistance to a battery of antibiotics including Ampicillin, Erythromycin, Oxytetracycline, Penicillin, Tetracycline and Tylosin were found in *Staphylococcus aureus, Salmonella*, Fecal coliforms, and coliforms from air isolates.

1. INTRODUCTION

The World Health Organization defines a bacterial strain as antibiotic resistant when a genetic change causes it to tolerate an antibiotic concentration higher than the concentration, which inhibits development of most strains of the same species (Ferrando, 1975). This definition does not take into account if the offspring of such bacteria would be resistant. Takafuji (1977) used the definition that microbial resistance was the ability of a microbial cell and its progeny to survive and multiply under environmental conditions that would inhibit or destroy other organisms. This definition was rather vague so this study combines the two definitions to create one that is more suitable to the subject. This study defines antibiotic resistant as the ability of a microbe and its progeny to survive and multiply due to a genetic change that causes them to tolerate an antibiotic concentration that would inhibit or destroy microbes of the same species.

The overall purpose of this study was to determine the presence of bacteria in the air surrounding and inside of several swine production and confinement facilities in the American Mid-West, and to determine if the isolated bacteria were resistant to antibiotics. It was the goal of this study to analyze and interpret the presence of antibiotic resistant bacteria in aerosol form. Swine production facilities generally confine a large amount of animals and give them a number of antibiotics. The microbial sampling was done to simulate human respiration to gain an understanding of the types and numbers of bacteria and fungi that a human would be exposed to in

the proximity to the facilities. Simulation of human respiration was accomplished through the use of two-stage Andersen Air samplers. The isolated airborne bacteria were then examined for their resistance to antibiotics using all applicable standard methods.

In the United States alone, hospital costs for managing the emergence of antimicrobial resistant bacteria have been estimated to be between one hundred million and ten billion dollars a year. It was stated in the United States National Swine Survey that more than twenty-five percent of all animal feed in the United States was above the recommended levels for antibiotic additives (Khachatourians, 1998).

The gut flora of the average, non-hospitalized human generally contains only 0.2% of antibiotic resistant *Escherichia coli* in the entire *Escherichia coli* gut population. (Linton, 1984). Over sixty percent of the entire populations of gut flora of *Escherichia coli* were found to be antibiotic resistant in calves, swine, and poultry that were treated with antibiotics to promote growth. This resistance was due to the oral use of antibiotics in animal confinement operations (Mathew, 1998). Modern animal husbandry techniques generally use large-scale confinement and animal concentration operations as well as the additions of antibiotics in feed in an effort to increase product yields (Scarpino and Quinn 1998).

Aerosolized antibiotic resistant bacteria could be spread over greater distances, and would be more difficult to contain than similar types of bacteria that were bound by another matrix such as soil or water. These bacteria would be harder to contain, that aspect alone would make them a much greater threat to the human population. The mode of infection by inhalation is much more effective than the common route of infection by general physical contact with the infected host (Dutkiewicz, 1994). Experiments with swine have shown that when ten billion colony-forming units (CFUs) of *Staphylococcus aureus* were ingested that the lymph nodes were testing positive for the organisms

within eight hours, and that the organisms were detectible in the animals' feces after twenty-four hours (Berends et al, 1996).

It was estimated that anywhere between 40% and 60% of all antibiotics currently manufactured for the United States were used in animals. About 90% of antibiotics used in animals, was added directly to animal feed to promote growth. This was lower than a medicinal dose or subtherapeutic dose of broad-spectrum antibiotics that was supposed to prevent disease and increase the rate of animal weight gain. This was a cheaper way to keep the animals healthy and to allow them to gain weight quicker than animal feeds alone. These treated animals generally gain weight about 4% to 5% faster than other animals (Dunlop et al, 1998).

This practice of feeding animals antibiotics has been encouraging the development and of antibiotic resistant bacteria within these animals. This was stated during a comparison of the poultry, swine, and the cattle industries for forms of antibiotic resistant bacteria with that of the human population (Threlfall et al, 1993). The use of antibiotics was prevalent in the swine and cattle industries as well as human medicine, but was not evident in the poultry industry in their study sampling. The cattle and swine industry showed both a marked increase in microbial antibiotic resistance as well as multiple resistance factors, while the poultry industry showed no marked increase in either (Threlfall et al, 1993). The human population also showed an increase in microbial resistance that mirrored the resistance found in the food animals (Threlfall et al, 1993). This suggested that the resistance factors were passed through the microbial contamination of the food that people ingest.

In a number of European nations we have seen a reduction in the use of antibiotics in the fish farming or the aquaculture industry. This industry has been successfully using immunization instead of antimicrobials to maintain the health of their product. The Swedish had also made progress in the

reduction of antimicrobials in their food-animal industries. Sweden became the first nation to ban completely the use of antibiotics as growth promoters in 1986. In 1998, the government of Denmark followed Sweden's lead by also enacting a similar ban on antibiotics (McGeer, 1998). They enhanced the rearing process by allowing the animals more space and more comfortable surroundings to offset the initial rise in mortality that occurred within their swine and poultry industries when the reduction in antibiotics first came into effect (Wise et al, 1993).

Impacts on Human Health

Fear of antibiotic resistant bacteria was that they could be transported to the human population, and render current medical treatments obsolete. We have seen evidence of this, an experiment done in Germany showed interesting results when they compared antibiotic resistant factors from two separate towns (Hummel et al, 1986). Both towns in question had a swine farm, but one town did not give antibiotics to the swine as growth factors while the other did. Neither town had a history of antibiotic resistance to the nourseothricin antibiotic that was being tested prior to its use as a growth promoter. After two years of adding nourseothricin to swine feed to promote growth they discovered a plasmid mediated resistance to nourseothricin antibiotics in the town where the swine were being medicated but not in the other town. They found resistance in *Escherichia coli* from swine, workers on the swine farm (from feces), and in the family members of those that worked in the swine farm. However, the resistance was also discovered in bacteria that were commonly found within humans that were without any connection to the swine farm or anyone connected to the swine farm. The resistance was also discovered in the bacteria that caused the urinary tract infections of those that lived in the town (Hummel et al, 1986). Although these bacteria were found in much smaller amounts in the humans their presence speaks volumes about their ability to be transferred to humans.

Infectious diseases are still one of the most common causes of death worldwide, and antibiotics or antimicrobials are the leading treatment method (McGeer, 1998). If we were to lose the use of antibiotics for treating infectious disease, the global cost in human life would be staggering. At a local level, areas surrounding swine production facilities might notice a rise in the difficulty of treating human health problems (Haglind, 1987). These problems would run from relatively minor to life threatening including, but not be limited to the following: respiratory problems, infectious disease, allergic and irritant responses, and hypersensitive reactions (DuPont, 1987 and Burge, 1997). All of these incidents would be prevalent among those in proximity and downwind of the facility that was distributing the antibiotic resistant bacteria (Scarpino and Quinn, 1998). These problems would occur in the absence of antibiotic resistance; however, antibiotic resistant pathogens would make clinical treatment more difficult.

When bacteria reproduce they are generally passed through the animals, and released in their feces. The land application of this manure could distribute the bacteria by placing them over a greater area. The microbes could be released from the dried feces while the animals were walking. This process is easier in the close quarters of the swine confinement area where the feces are being stirred up by the animals walking or routing through them. Once these microbes are in the air of the swine production they could be released to the outside through the facilities exhaust fans or by the containment unit's natural airflow. Most communities complain about the odors that emanate from these swine production facilities, and it is a safe assumption that there is most likely some health risks that accompanies these odors.

It is probable that antibiotic resistant bacteria existed prior to the widespread use of antibiotics in small populations that had no great selective advantage over normal bacteria. It is also likely that these resistant bacteria were a result of a genetic mutation or that they came into existence

as the result of a normal genetic event such as translation, translocation, transformation, or transduction. No matter how they came about, their existence has been promulgated by the excess presence of antibiotics in the animal feed. This animal feed acts as a selective agent to further the success of antibiotic resistant bacteria.

It has been shown that bacteria can move from livestock to humans when both were in close or direct contact to one another. This has been demonstrated in multiple case studies including a paper by Nijsten (1996) that found the same antibiotic resistant *Escherichia coli* in both the feces of swine and swine farmers. They inferred that this resistance was developed in the swine due to the presence of the antibiotics, and was transferred horizontally to the farmers from contact with the animals' feces (Nijsten, 1996).

2. MATERIALS AND METHODS

Site Selection

The sites for this study were both located in the rural Midwest of the United States. The sites were not near any heavily populated areas, and were miles from any other swine production facilities. These sites were both sampled with the full cooperation of the operator of the facility. The samples were taken from inside the facilities upwind and downwind of the facility. Each of the sites was sampled on two separate occasions and replicates were taken of each sample.

The first site, designated SITE A, was a facility that housed one thousand swine for finishing purposes before the animals were sent to market. The building was 2 years old with dimensions 12 m wide by 60 m long by 3 m high. The sides of the facility were concrete to 1 m, but above one 1 m were comprised of mesh to allow the easy exchange of air. Shades were located above the mesh and were controlled automatically depending on the temperature inside the facility. The facility had a

grated floor that allowed the waste material to fall through and collect in a large pit beneath the building. The pit itself was 1.3 meters deep and the length and width of the building. The pit was emptied twice a year, and contents injected into cropland surrounding the confinement facility as a source of nutrients. This facility had a tunnel ventilation system that used five fans that were 1m in diameter and located at the East side of the facility to draw air through the building. This system was primarily used to cool the swine and maintain the temperature of the building.

The Second site, designated SITE B, was a facility that housed eleven hundred swine for finishing purposes before the animals were sent to market. The building was 4 years old with buildings dimensions 12 m wide by 60 m long by 3 m high. The sides of the facility were concrete to 1 m, but above one 1 m were comprised of mesh to allow the easy exchange of air. Shades were located above the mesh and were controlled automatically depending on the temperature inside the facility. It had a grated floor that allowed the waste material to fall through and collect in a large pit beneath the building. The pit was 1.3 meters deep and the length and width of the building. The pit was emptied twice a year, and contents injected into cropland surrounding the confinement facility as a source of nutrients. This facility had a chimney ventilation system that used vents along the length of the roof of the facility to draw air through the sides of the building and up through the roof. This system was used to cool the swine and maintain the temperature of the building.

An oral survey was given to each of the operators to help the researchers prepare for this study, and provide an overview of the facilities operations. These surveys were used to determine the types and quantities of the subtherapeutic doses of antibiotics used on the swine at each facility. The answers to these questions provided the number of animals housed in each building, the type of ventilation systems used in each building, and how the operator handled the waste from the facilities.

When at the site, wind direction and speed was noted, and the sampling sites were inside, 25 meters upwind, and downwind of the facility. Each of the samples was taken from atop of a 1.3 meter high tri-pod to simulate the height of the average person. The Andersen Two-Stage Viable Microbial Particle Sizing Sampler Instrument was used to collect bacterial and fungal samples from the animal confinement facilities. The sampler was loaded with plates of media that were conducive to culturing the types of organisms that were to be isolated. This study used Malt Extract agar (Difco Laboratories, Detroit, MI) for fungi and Tryptic Soy Agar (Difco Laboratories, Detroit, MI) for the isolation of bacterial species. The Andersen Two-Stage Sampler is a cascade impactor that allows the air to be drawn into and through the sampler by a pump at a rate of 0.02832 m^3/min. The two stages allow for the separation of the particles according to their size. The result was that the non-respirable particles which were roughly 0.8 um or larger were deposited on the first or top Petri dish and the respirable particles of 0.8 um or smaller were deposited on the second or bottom Petri dish. There was some overlapping of particles of similar size between the two stages. Separate equipment including a pump and Andersen Two-Stage Sampler were used for each location on the site, and only one site was sampled per trip to prevent cross contamination. The samples were taken in triplicate for an amount of time determined to provide roughly 30 Colony Forming Units (CFUs) per Plate. The colonies that developed were counted after 24 and 48 hrs to determine heterotrophic plate count prior to the Replica Plate Method.

The Replica Plate Method was used to isolate the colonies of the types of microbes that were to be used to determine antibiotic resistance. They were all done in triplicate to insure statistical significance. The media that were used were chosen since they were known to encourage the growth of the microbial strains that were to be examined, and discourage the growth of less desirable microbial species that were not to be examined: Brilliant Green Agar (BGA) for differentiating

salmonella, Tryptic Soy Agar (TSA) as a control, Chapman Stone Medium (CSM) for differentiating staphylococci, MacConkey Agar (MCA) for the specific purpose of isolating and differentiating lactose fermenting gram-negative enteric bacilli such as coliforms, and m-Fecal Coliform Agar (m FC) for isolating and differentiating fecal coliforms. All agars were acquired from Difco Laboratories in Detroit, MI. All organisms were identified using a microscope and their known morphological characteristics. *Staphylococcus aureus* was further confirmed using Bacto Coagulase Plasma **(Fisher Scientific)**. This allowed for the identification of the organisms that would be tested for antibiotic resistance. Then several organisms from each plate were transferred onto TSA slants to created cultures to be used for the Kirby-Bauer Disc Diffusion Method.

Kirby-Bauer Disc Diffusion Method was the methods used to determine antimicrobial susceptibility to antibiotics. The organisms were analyzed using the United States National Committee for Clinical Laboratory Standards (NCCLS) *Approved Standard M2-A6* (1997) and several other sources. The following was a description of the method itself. Three Mueller-Hinton Agar (MHA) plates and three TSA were used for each of the organisms to be tested for antibiotic resistance. Six types of Antibiotic Susceptibility Test Discs (Difco Laboratories, Detroit, MI) were used in the Kirby-Bauer method. Tylosin at a concentration of 6 mcg and Oxytetracycline at a concentration of 30 mcg were both used because they were antibiotics that the farmers had indicated that they used in the swine. Tetracycline at a concentration of 30 mcg was used because it was in the antibiotic family, Tetracycline, along with Oxytetracycline. Erythromycin at a concentration of 15 mcg was used because it was in the antibiotic family, Macrolides, along with Tylosin. Ampicillin at a concentration of 10 mcg and Penicillin G at a concentration of 10 mcg were both used because they were in the antibiotic family, Penicillins, which is commonly used in human medical treatment. This was also done for the TSA plates. This was repeated three times for each of the organisms to be

tested. All of the plates were then incubated at 35°C for 24 hours. The plates were then checked for susceptibility after 24 hours in the following manner. Calipers were used to measure the entire zone of inhibition. The zones were recorded for all of the plates and then compared with the NCCLS standards. A determination was then made as to whether the organism was susceptible, intermediately susceptible, or resistant.

Control organisms were used to validate that the proper types of microorganisms would grow on the appropriate selective media and to validate the effectiveness of the antibiotics. These organisms were chosen along United States National Committee for Clinical Laboratory Studies (NCCLS) guidelines. Control organisms for the media were obtained from the Cultures in the Environmental Microbiology Laboratory at the Shriner's Burn Center in Cincinnati, Ohio. Control organisms for the antibiotics were used to validate that the test system was working for each antibiotic. These organisms were chosen along NCCLS guidelines.

3. RESULTS

The statistical averages per site at each sampling point (Inside, Upwind, and Downwind) will be presented for both the number of Colony forming units/cubic meter ($CFUs/m^3$) of air recovered, and the percent of resistant, susceptible, and intermediate microorganism that were isolated and tested from those recovered at each site. The identification of the organisms isolated for both fungus and bacteria will also be stated.

Number of Organisms at Each Site

The average amount of CFUs/m^3 was determined for each site at each sampling point for both fungi and bacteria. These were further separated into the top plate or non-respirable organisms, and the bottom plate that contains the respirable organism. TSA and MEA plates were used for the initial collections of organisms. The data, for each site, is presented in Tables 1 and 2.

Table 1: Average CFUs/m^3 for all visits to Site A

Type of agar, location on site, place in sampler	CFUs/m^3
Site A	
Bacteria-Inside-Nonrespirable	31,061
Bacteria -Inside- Respirable	7,345
Bacteria -Upwind- Nonrespirable	13
Bacteria -Upwind- Respirable	59
Bacteria -Downwind- Nonrespirable	2,810
Bacteria -Downwind- Respirable	1,014
Fungi-Inside- Nonrespirable	635
Fungi -Inside- Respirable	81
Fungi -Upwind- Nonrespirable	0
Fungi -Upwind- Respirable	0
Fungi -Downwind- Nonrespirable	3
Fungi -Downwind- Respirable	6

Table 2: Average CFUs/m^3 for all visits to Site B

Type of agar, location on site, place in sampler	CFUs/m^3
Site B	
Bacteria -Inside- Nonrespirable	8,312
Bacteria -Inside- Respirable	2,052
Bacteria -Upwind- Nonrespirable	5
Bacteria -Upwind- Respirable	38
Bacteria -Downwind- Nonrespirable	2,087
Bacteria -Downwind- Respirable	607
Fungi -Inside- Nonrespirable	16
Fungi -Inside- Respirable	9
Fungi -Upwind- Nonrespirable	0
Fungi -Upwind- Respirable	0
Fungi -Downwind- Nonrespirable	4
Fungi -Downwind- Respirable	7

Organism Identification

The following fungi were identified from inside site A: *Acremonium, Alternaria, Aspergillus niger, Candida, Chrysosporium, Monilia, Mortierella,* and *Mucor* from inside the facility at site A. The bacteria that were recovered from inside the facility at site A were: Coliforms, Fecal Coliforms, *Salmonella sp.*, and *Staphylococcus aureus*.

Chrysosporium was the only fungus found upwind of the facility at site A. The bacteria that were recovered from upwind of the facility at site A were: *Fecal Coliforms* and *Salmonella*. There were also organisms that could not be identified.

The following fungi were identified from downwind of site A: *Alternaria, Chrysosporium,* and *Mucor* from downwind of the facility at site A. The bacteria that were recovered from downwind of the facility at site A were: Coliforms, Fecal Coliforms, *Salmonella sp.*, and *Staphylococcus aureus*.

The following fungi were identified from inside of site B: *Alternaria, Aspergillus fumigatus, Aspergillus niger, Candida, Chrysosporium,* and *Rhizopus* from inside the facility at site B. The bacteria that were recovered from inside the facility at site B were: Coliforms, Fecal Coliforms, *Salmonella sp.*, and *Staphylococcus aureus*.

Chrysosporium and *Rhizopus* were the only fungus found upwind of the facility at site B. The bacteria that were recovered from upwind of the facility at site B were: *Staphylococcus aureus* and *Salmonella sp*.

The following fungi were identified: *Candida* and *Chrysosporium* from downwind of the facility at site B. The bacteria that were recovered from downwind of the facility at site B were: *Coliforms*, *Salmonella sp.*, and *Staphylococcus aureus*.

Antibiotic Resistance

The antibiotic resistance to six different antibiotics (Ampicillin, Erythromycin, Oxytetracycline, Penicillin, Tetracycline, and Tylosin) was determined for a representative sample of each of the organisms recovered from each of the sampling points at each site for each visit to that site. That data was then combined to give an average resistance for the site for comparison between each sampling point (Inside, Upwind, and Downwind).

Site A

The bacteria from Site A were found to have the following reactions to Ampicillin. The *Staphylococcus aureus* located on the inside of the facility were 90% resistant and 10% susceptible, upwind none were recovered, and downwind they were 80% resistant and 20% susceptible. The *Salmonella* located on the inside of the facility were 90% resistant and 10% intermediate, upwind they were 100% susceptible, and downwind they were 20% resistant and 80% susceptible. The Fecal Coliforms located on the inside of the facility were 90% resistant and 10% intermediate, upwind they were 100% intermediate, and downwind they were 80% resistant and 20% susceptible. The Coliforms located on the inside of the facility were 60% resistant, 30% intermediate, and 10% susceptible, upwind none were recovered, and downwind they were 100% resistant.

The bacteria from Site A were found to have the following reactions to Erythromycin: The *Staphylococcus aureus* located on the inside of the facility were 100% resistant, upwind none were recovered, and downwind they were 80% resistant and 20% susceptible. The *Salmonella* located on the inside of the facility were 100% resistant, upwind they were 100% susceptible, and downwind they were 80% resistant and 20% susceptible. The Fecal Coliforms located on the inside of the facility were 90% resistant and 10% intermediate, upwind they were 100% resistant, and downwind they were 80% resistant and 20% susceptible. The Coliforms located on the inside of the facility were 90% resistant and 10% intermediate, upwind none were recovered, and downwind they were 100% resistant.

The bacteria from Site A were found to have the following reactions to Oxytetracycline: The *Staphylococcus aureus* located on the inside of the facility were 90% resistant and 10% susceptible, upwind none were recovered, and downwind they were 80% resistant and 20% susceptible. The *Salmonella* located on the inside of the facility were 100% resistant, upwind they were 100%

susceptible, and downwind they were 80% resistant and 20% susceptible. The Fecal Coliforms located on the inside of the facility were 80% resistant and 20% intermediate, upwind they were 100% resistant, and downwind they were 80% resistant and 20% susceptible. The Coliforms located on the inside of the facility were 70% resistant and 30% susceptible, upwind none were recovered, and downwind they were 100% resistant.

The bacteria from Site A were found to have the following reactions to Penicillin: The *Staphylococcus aureus* located on the inside of the facility were 90% resistant and 10% susceptible, upwind none were recovered, and downwind they were 100% resistant. The *Salmonella* located on the inside of the facility were 20% resistant and 80% intermediate, upwind they were 100% susceptible, and downwind they were 20% intermediate and 80% susceptible. The Fecal Coliforms located on the inside of the facility were 40% resistant, 50% intermediate, and 10% susceptible, upwind they were 100% intermediate, and downwind they were 80% resistant and 20% susceptible. The Coliforms located on the inside of the facility were 40% resistant, 50% intermediate, and 10% susceptible, upwind none were recovered, and downwind they were 100% resistant.

The bacteria from Site A were found to have the following reactions to Tetracycline: The *Staphylococcus aureus* located on the inside of the facility were 80% resistant and 20% susceptible, upwind none were recovered, and downwind they were 80% resistant and 20% susceptible. The *Salmonella* located on the inside of the facility were 90% resistant and 10% intermediate, upwind they were 100% susceptible, and downwind they were 80% resistant and 20% susceptible. The Fecal Coliforms located on the inside of the facility were 80% resistant and 20% susceptible, upwind they were 100% intermediate, and downwind they were 80% resistant and 20% susceptible. The Coliforms located on the inside of the facility were 60% resistant, 10% intermediate, and 30% susceptible, upwind none were recovered, and downwind they were 100% resistant.

The bacteria from Site A were found to have the following reactions to Tylosin: The *Staphylococcus aureus* located on the inside of the facility were 100% resistant, upwind none were recovered, and downwind they were 80% resistant and 20% susceptible. The *Salmonella* located on the inside of the facility were 90% resistant and 10% intermediate, upwind they were 100% susceptible, and downwind they were 80% resistant and 20% susceptible. The Fecal Coliforms located on the inside of the facility were 80% resistant and 20% susceptible, upwind they were 100% susceptible, and downwind they were 80% resistant and 20% susceptible. The Coliforms located on the inside of the facility were 100% resistant, upwind none were recovered, and downwind they were 100% resistant.

Site B

The bacteria from Site B were found to have the following reactions to Ampicillin. The *Staphylococcus aureus* located on the inside of the facility were 85% resistant and 15% susceptible, upwind they were 100% susceptible, and downwind they were 80% resistant and 20% susceptible. The *Salmonella* located on the inside of the facility were 11% resistant, 56% intermediate, and 33% susceptible, upwind they were 100% susceptible, and downwind they were 15% resistant, 47% intermediate, and 38% susceptible. The Fecal Coliforms located on the inside of the facility were 100% resistant, upwind none were recovered, and downwind none were recovered. The Coliforms located on the inside of the facility were 33% resistant, 33% intermediate, and 33% susceptible, upwind none were recovered, and downwind they were 100% susceptible.

The bacteria from Site B were found to have the following reactions to Erythromycin: The *Staphylococcus aureus* located on the inside of the facility were 100% resistant, upwind they were 100% resistant, and downwind they were 95% resistant and 5% susceptible. The *Salmonella* located on the inside of the facility were 94% resistant and 6% susceptible, upwind they were 100%

susceptible, and downwind they were 100% resistant. The Fecal Coliforms located on the inside of the facility were 100% resistant, upwind none were recovered, and downwind none were recovered. The Coliforms located on the inside of the facility were 63% resistant and 33% intermediate, upwind none were recovered, and downwind they were 100% resistant.

The bacteria from Site B were found to have the following reactions to Oxytetracycline: The *Staphylococcus aureus* located on the inside of the facility were 100% resistant, upwind they were 100% resistant, and downwind they were 95% resistant and 5% susceptible. The *Salmonella* located on the inside of the facility were 89% resistant and 11% susceptible, upwind they were 100% susceptible, and downwind they were 93% resistant and 7% susceptible. The Fecal Coliforms located on the inside of the facility were 50% resistant and 50% susceptible, upwind none were recovered, and downwind none were recovered. The Coliforms located on the inside of the facility were 66% intermediate and 33% susceptible, upwind none were recovered, and downwind they were 100% resistant.

The bacteria from Site B were found to have the following reactions to Penicillin: The *Staphylococcus aureus* located on the inside of the facility were 90% resistant and 10% susceptible, upwind they were 100% susceptible, and downwind they were 80% resistant and 20% susceptible. The *Salmonella* located on the inside of the facility were 56% intermediate and 44% susceptible, upwind they were 100% susceptible, and downwind they were 54% intermediate and 46% susceptible. The Fecal Coliforms located on the inside of the facility were 100% susceptible, upwind none were recovered, and downwind none were recovered. The Coliforms located on the inside of the facility were 66% intermediate and 33% susceptible, upwind none were recovered, and downwind they were 100% susceptible.

The bacteria from Site B were found to have the following reactions to Tetracycline: The *Staphylococcus aureus* located on the inside of the facility were 100% resistant, upwind they were 100% resistant, and downwind they were 95% resistant and 5% susceptible. The *Salmonella* located on the inside of the facility were 89% resistant and 11% susceptible, upwind they were 100% susceptible, and downwind they were 93% resistant and 7% susceptible. The Fecal Coliforms located on the inside of the facility were 50% resistant and 50% intermediate, upwind none were recovered, and downwind none were recovered. The Coliforms located on the inside of the facility were 100% resistant, upwind none were recovered, and downwind they were 100% resistant.

The bacteria from Site B were found to have the following reactions to Tylosin: The *Staphylococcus aureus* located on the inside of the facility were 95% resistant and 5% susceptible, upwind they were 100% resistant, and downwind they were 95% resistant and 5% susceptible. The *Salmonella* located on the inside of the facility were 78% resistant and 22% susceptible, upwind they were 100% susceptible, and downwind they were 93% resistant and 7% susceptible. The Fecal Coliforms located on the inside of the facility were 100% resistant, upwind none were recovered, and downwind none were recovered. The Coliforms located on the inside of the facility were 66% resistant and 33% susceptible, upwind none were recovered, and downwind they were 100% intermediate.

4. DISCUSSION AND CONCLUSION

Enumeration of Microorganisms

This study found levels of microorganisms above 10^3 CFUs/m^3 of air for both inside and downwind of the swine producing facilities that were sampled. These levels were high for both the respirable and non-respirable microorganisms. Scarpino and Quinn, 1998, used levels at or above

10^3 CFUs/m^3 as an indicator of high bacterial conditions. These conditions were also found to be high enough that Attwood et al, 1987, observed adverse health effects in farm workers. These adverse health effects were found in surveys by Donham et al, 1989 to include, but not be limited to increased occurrences of respiratory symptoms, more frequent colds, chest illness, and a history of pneumonia. The individuals in those two studies were farm workers who were often in close proximity to the facilities during the workday. These respiratory symptoms occurred four to eight hours after exposure in 14% of cases according to Bongers et al, 1987. Prolonged exposure to these high levels of bacteria and fungi could lead to even more adverse health effects for both those that work in these types of facilities and those that live in close proximity to them.

Types of Microorganisms

The quantity of microorganisms being released from the swine producing facilities has been shown to be large enough to constitute a problem. It is necessary to examine the types of individual organisms to explore what problems, if any, these organisms could have for humans. Some of the organisms that were identified are known human pathogens and therefore are of a greater concern.

The bacteria that were identified that pose a potential health risk include *Staphylococcus aureus* and *Salmonella. Staphylococcus aureus* is an organism that is known to produce a number of infections in both humans and animals including skin infections. It is not only a potential threat to humans but also other livestock that might be located downwind. *Salmonella* is also known to be both a human and animal pathogen. Fecal coliforms, such as *Escherichia coli,* were also recovered from the facilities. These organisms are also known pathogens, but none were positively identified in this study. However, they have an even greater potential to harm humans. In the past few years quite a few deaths related to food poisoning have been attributed to fecal coliforms such as *Escherichia coli 0157:H7.*

The fungi that were identified in this study are also quite interesting. *Alternaria, Aspergillus, Candida, Monilia, Mucor,* and *Rhizopus* are all types of fungi that were identified in this study that are know to have adverse human health effects (Scarpino and Quinn, 1998). *Alternaria* is known to cause chronic sinusitis, ulcerated cutaneous infections, and are agents of onychomycosis. Candida is a known human pathogen. Monilia rarely causes human infection, but is an irritant to the respiratory system. The *Aspergillus* genus can cause a disease known as aspergillosis. *Aspergillus, Mucor, and Rhizopus* are all opportunistic invaders that can have devastating effects on those individuals with suppressed immune systems.

Antibiotic Resistance

The resistance patterns within this study show that the microorganisms that are being released from the swine confinement facilities do carry resistance to a variety of antibiotics. The majority of those microorganisms found inside and downwind of the facilities shows resistance. The majority of the microorganisms located upwind of the facilities showed susceptibility to the antibiotics evaluated in this study. The few cases of microorganisms found upwind that showed resistance could be a result of minor variations in the wind direction during the sampling period.

Staphylococcus aureus was the only microorganism taken to species. It showed the greatest instances of resistance to the broad spectrum of antibiotics. Scarpino and Quinn, 1998, obtained similar results. They found that many of the *Staphylococcus* isolates from residences near swine production facilities showed resistance to one or more antibiotics, and several of the organisms were able to grow well in the presence of all of the antibiotics. The *Staphylococcus aureus* isolated in this study seemed to be able to consistently grow well in the presence of most or all of the evaluated antibiotics. *Staphylococcus aureus* resistant to antibiotics has been seen in other studies as well

(Pereira, 1995). The other three types of microorganisms all consistently showed resistance to more than one type of antibiotic.

The microorganisms were generally resistant to Oxytetracycline and Tylosin, which are the antibiotics that are used on the farms. The same was true for the two antibiotics that were in the same classes, which were Tetracycline and Erythromycin. The microorganisms were generally resistant to both Ampicillin and Penicillin, the two broad-spectrum antibiotics used in this study. The percentages of resistance were not as defined for Ampicillin and Penicillin as they were for the other antibiotics in the study, which the animals are exposed to daily.

The conclusions reached by this study were straightforward. The use of antibiotics in swine confinement facilities was producing a number of microorganisms that are antibiotic resistant. The large numbers of microorganisms within the air are known to have adverse human health effects. Antibiotic resistant organisms are also known to have adverse human health effects. It seems prudent that for the safety of the farm workers, and those that live in proximity to the facilities that some changes within these types of facilities need to be made.

The easiest change to make is that anyone working inside the facility should wear a respirator to insure that they are not overly exposed to the microorganisms. The placement of these types of facilities must be chosen carefully. It would be logical to place these facilities in areas that do not have a large population living within a close proximity to protect those around the facility. Special attention should also be paid to the prevailing winds in the area. Placing them in areas with low wind that generally do not have a large population downwind could decrease the impact of these facilities. The types of antibiotics used in the facility could be placed on a rotation in consultation with a veterinarian. The antibiotics from different classes could be used to accomplish the same task without allowing the organisms long time periods to promulgate their resistance to future

generations. There is also the possibility that some control devices be placed into the facilities in order to limit the viability of the microorganism coming off of the facility. This could be UV lights or HEPA filters in the exhaust fans. However, this seems to be both very costly and may not produce a large amount of noticeable effects with the current technology and facility designs.

References

Attwood, P. et al. (1987) A Study of the Relationship Between Airborne Contaminants and Environmental Factors in Dutch Swine Confinement Buildings. *American Industrial Hygiene Association Journal,* **48**(8); 745-751.

Berends, B.R., H.A.P. Urlings, J.M.A. Snijders, F. Van Knapen. (1996) Identification and quantification of risk factors in animal management and transport regarding Salmonella spp. in swine. *International Journal of Food Microbiology,* **30**; 37-53.

Bongers, P. et al. (1987) Lung Function and Respiratory Symptoms in Swine Farmers. *British Journal of Industrial Medicine,* **44**; 819-823.

Burge, S. (1997) Respiratory symptoms. *Occupational Medicine,* **47**; 55-56.

Donham, K. et al. (1989) Environmental and Health Studies of Farm Workers in Swedish Swine Confinement Buildings. *British Journal of Industrial Medicine,* **46**; 31-37.

Dunlop, R.H., S.S. McEwen, A.H. Meek, R.C. Clarke, W.D. Black, and R.M. Friendship. (1998) Associations among antimicrobial drug treatments and antimicrobial resistance of fecal Escherichia coli of swine on 34 farrow-to-finish farms in Ontario, Canada. *Preventive Veterinary Medicine,* **34**; 547-577.

Dupont, H.L., J.H. Steele. (1987) Use of Antimicrobial Agents in Animal Feeds: Implications for Human Health. *Reviews of Infectious Diseases,* **9**; 447-449.

Dutkiewicz, J., Z. Pomorski, J. Sitkowska, E. Krysinska-Traczyk, C. Skorska, Z. Prazmo, G. Cholewa, and H. Wojtowicz. Airborne microorganisms and endotoxin in animal houses. *GRANA,* **33**; 85-90.

Ferrando, R. Future of additives in animal feeding. *World Review of Nutrition and ietetics,* **22**; 183-235.

Haglind P. and R. Rylander. (1987) Occupational exposure and lung function measurements among workers in swine confinement buildings. *Journal of Occupational Medicine,* **29**; 904-907.

Hummel, R., H. Tschape, and W. Witte. (1986) Spread of plasmid-mediated nourseothricin resistance due to antibiotic use in animal husbandry. *Journal of Basic Microbiology,* **8**; 461-466.

Khachatourians, George G., BA, MA, PHD. (1998) Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. *Canadian Medical Association Journal,* **159**; 1129-1136.

Linton, A.H. (1984) Antibiotic-resistant bacteria in animal husbandry. *British Medical Bulletin,* **40**; 91-95.

Mathew, A.G., W.G. Upchurch, and S.E. Chattin. (1998) Incidence of Antibiotic Resistance in Fecal *Esherichia coli* Isolated from Commercial Swine Farms. *Journal of Animal Science*, **76**; 429-434.

McGeer, Allison J. (1998) Agricultural antibiotics and resistance in human pathogens: villain or scapegoat? *Canadian Medical Association Journal,* **159**; 1119-1120.

Nijsten, R., N. London, A. van den Bogaard and E. Stobberingh. (1996) Antibiotic resistance among Escherichia coli isolated from fecal samples of swine farmers and swine. *Journal of Antimicrobial Chemotherapy,* **37**; 1131-1140.

Pereira, M.S.V., J.P. Siqueira-Junior (1995) Antimicrobial drug resistance in *Staphylococcus aureus* isolated from cattle in Brazil. *Letters in Applied Microbiology,* **20**; 391-395.

Scarpino, P.V., and H. Quinn. (1998) Bioaerosol distribution patterns adjacent to two swine-growing-finishing housed confinement units in the American Midwest. *Journal of Aerosol Science,* **29**; S553-S554.

Scarpino, P.V., and H. Quinn. (1998) Bacterial and fungal bioaerosol emissions from a completely housed swine-growing-finishing operation. Presented at the June 22-26, 1998 17t^{h} Annual AAAR Conference. Cincinnati, Ohio. USA.

Takafuji, Earnest T. (1977) The effect of antibiotic drug resistance on the environment and its impact on public health. *Preventive Medicine,* **6**; 312-318.

Threlfall, E.J., B. Rowe, and L.R. Ward. (1993) A comparison of multiple drug resistance in salmonellae from humans and food animals in England and Wales, 1981 and 1990. *Epidemiology-and-Infection,* **111**; 189-197.

Wise, Richard et al. (1993) Antimicrobial resistance. *British Medical Journal*, **317**; 609-610.