Antibiotic Resistant Bacteria in Subsurface Tile Drainage from Manure Amended Fields

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Introduction

In order to prevent the spread of disease in animal feeding operations, antimicrobials often are added to animal feed or water. Tylosin and tetracycline are two of the most commonly used veterinary antibiotics in the swine industry. This multi-year study aims to track the transport of antibiotic resistant bacteria (ARB) from the land-applied manure to the subsurface water drainage systems, with consideration to the field management practices in place.

Materials and Methods

One-acre plots were sampled weekly for three years at Iowa State University's Northeast Research Farm near Nashua, Iowa. Each plot is outfitted with separate subsurface drainage lines with a monitoring station at the outlet where water samples were collected. Five total treatment combinations of manure application timing and tillage practices were sampled in triplicate for a total of 15 plots sampled each week whenever tiles were flowing. A summary of plot treatments sampled is available in Table 1.

Following collection, each sample was analyzed for ARBs by filtering the water sample and growing the resulting fecal indicator bacteria on mediums containing *Enterococcus* with either tetracycline, tylosin, or no antibiotics (control). After the incubation period the bacteria colonies that persisted were counted (colony forming units/100mL) and the data analyzed.

Results and Discussion

Enterococci were found frequently in water drainage samples throughout the sample collection season, although levels were highly variable across plot treatments and along the sampling season. Antibiotic resistant *Enterococci* (*Enterococcus* + Tetracycline, *Enterococcus* + Tylosin) were detected far less frequently, and when detection occurred, it was at lower concentrations. Table 2 shows this is true across all three years of the study.

There was high variability between years of sample collection in total bacteria concentrations. The first year of the study (2016) shows the highest overall median *Enterococcus* concentration reported at 6 CFU/100mL with the third year (2018) showing the second highest level at 5 CFU/100mL. In contrast, the second year of sample collection (2017) showed an overall *Enterococcus* concentration of only 1 CFU/100mL. This pattern is true for the resistant bacteria with 2016 having the most, followed by 2018 and 2017, but all concentrations were less than 1 CFU/100mL. Concentrations of tylosin resistant *Enterococcus* were low and no patterns between years could be established. Total flow through the system was greater in 2016 and 2018 than 2017 due to higher precipitation levels, which could help to explain the variability in total bacteria concentrations. Additionally, initial antibiotic levels given to

swine are hypothesized to be greater in 2016, before the Veterinary Feed Directive was implemented.

Over the entire three-year sampling period, plots receiving manure in the spring had the greatest median levels of *Enterococcus* detected, although the no manure control had the lowest at 7 CFU/100mL and 5 CFU/100mL, respectively. The highest three-year resistance bacteria concentration medians were found in the plots receiving manure in the late fall. However, the concentrations were overall low at less than 1 CFU/100mL for tetracycline resistance and tylosin resistance. Due to the sampling schedule, this was expected as sampling occurs during the summer drainage season, most closely following the spring manure application season. From year to year, the median bacteria concentrations are highly variable between manure application timing and year, as shown in Table 2.

Three-year median bacteria concentrations did not vary widely between plots receiving chisel plow tillage and plots with no tillage for either *Enterococcus* or antibiotic resistant *Enterococcus* (Table 3). These results suggest tillage practice does not have a significant impact on the transport of ARBs through the tile drainage system.

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Table 1. Summary of plot treatments at the Northeast Research Farm, Nashua, Iowa. Each management strategy will be sampled in triplicate.

Combination number	Manure application	Tillage	Crop
1	Fall	Chisel plow	Continuous corn
2	Early fall	No till	Rotational corn
3	Late fall	No till	Rotational corn
4	Spring	Chisel plow	Continuous corn
Control			Rotational corn

Table 2. Median bacteria concentrations detected by manure application time.*

Manure application time	*Enterococcus* (CFU/100mL)			*Enterococcus* + Tetracycline (CFU/100mL)			*Enterococcus* + Tylosin (CFU/100mL)		
	2016	2017	2018	2016	2017	2018	2016	2017	2018
Early fall	6.00	2.00	5.00	1.00	ND	ND	ND	ND	ND
Late fall	11.00	1.00	5.00	2.00	ND	ND	ND	ND	ND
Spring	9.00	3.00	7.00	1.00	ND	ND	ND	ND	ND
Control	5.00	ND	8.00	ND	ND	ND	ND	ND	ND
Total	7.00	1.00	6.00	ND	ND	ND	ND	ND	ND

***ND signifies a non-detect.**

Table 3. Median bacteria concentrations detected by tillage treatment.*

Tillage treatment	*Enterococcus* (CFU/100mL)			*Enterococcus* + Tetracycline (CFU/100mL)			*Enterococcus* + Tylosin (CFU/100mL)		
	2016	2017	2018	2016	2017	2018	2016	2017	2018
No till	6.00	2.00	5.00	ND	ND	ND	ND	ND	ND
Chisel plow	5.00	2.00	5.00	ND	ND	ND	ND	ND	ND
Total	6.00	2.00	5.00	1.00	ND	ND	ND	ND	ND

***ND signifies a non-detect.**