RESEARCH REPORT
PUBLIC HEALTH/WORKER SAFETY

Title: Monitoring of Antimicrobial Resistance in Food Animal Production: Partnership between National Antimicrobial resistance Monitoring System (NARMS) and Academia – **NPB #11-175** revised

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Industry Summary:

Collaborative partners including four Universities (Ohio State, Minnesota, North Carolina State and Iowa State), industry and federal agency (USDA) conducted a one year on-farm monitoring project in swine production systems focusing on four major swine producing states: Iowa, North Carolina, Minnesota and Ohio. The one-year monitoring project included four pathogens of pork safety significance including *Salmonella, Campylobacter, Escherichia coli* and *Enterococcus spp*. The main emphasis of the project was to understand and monitor antimicrobial resistance among these organisms and develop a representative sampling scheme that may help build a national on-farm monitoring program. The study documented the common occurrence of the organisms at various proportions in all four states. While this is expected and not surprising, there was a great deal of variation on serotypes and antimicrobial resistance patterns.

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Keywords: include at least 5 keywords

Antimicrobial resistance monitoring, swine, Salmonella, Campylobacter, E coli, Enterococcus

Scientific Abstract

A pilot on-farm antimicrobial resistance monitoring program on four pathogens of pork safety significance (*Salmonella, Campylobacter, Escherichia coli* and *Enterococcus* spp*)* in partnership with university, industry and federal agency (USDA) was conducted in swine production systems located in four major swine producing states: Iowa, North Carolina, Minnesota and Ohio. A total of 4,426 fresh fecal samples from 148 barns from the four states were collected and shipped to USDA-ARS BEAR laboratory on the same day of collection for an overnight delivery. Each fecal sample was examined for the presence/absence of *Campylobacter, E. coli,*

Enterococcus, Salmonella as well as MRSA and the isolates were tested for antimicrobial resistance. The prevalence of the pathogens varied from 78%-89% (*Campylobacter*), 97.8%-99.5% (*E. coli*) and 3.3%-12.4% (*Salmonella*) among the four locations. The proportion of antimicrobial resistant foodborne (*Salmonella* and *Campylobacter*) and commensal organisms (*E. coli* and *Enterococcus*) recovered from the study locations was relatively high and various antimicrobial resistance patterns were detected. Multidrug resistance (MDR) was very common among foodborne as well as commensal organisms included in this pilot study. The one-year monitoring project will be very helpful in understanding and monitoring antimicrobial resistance among major foodborne and commensal pathogens and develop a representative sampling scheme that may help build a national on-farm monitoring program.

Introduction

Although antimicrobial use may result in bacteria (including both food borne and commensals) that are resistant, the exact fate of these populations in terms of persistence and transmission in the host/environment has been difficult to determine. Antimicrobial use patterns in animal production (therapeutic versus growth promotion) and agriculture in general, further complicate the resistance picture. Additionally, while transmission of resistant bacteria from animals to humans occurs, it has been difficult to assess the frequency and extent to which this occurs, and the impact transmission of resistant bacteria or resistance genes has on human health. National and international debate has escalated over these issues. In the U.S., experts have been unable to reach consensus regarding the impact of AR in animal production and its impact on human health. However, by 2006 the European Union, invoking the precautionary principle, banned the use of antimicrobials for growth promotion. Consequently, there is a critical need to define the extent of AR in food animal production and elucidate the factors favoring the development or acquisition of AR. Further, tracking of antimicrobial resistant microorganisms through production systems using highly discriminatory phenotypic and genotypic methods will provide critical information when food related outbreaks occur.

Monitoring for the development of antimicrobial resistance in human medicine has been in place since antimicrobials first became available, however, it was primarily limited to hospital programs. As resistance to new antimicrobials emerged, and multiple drug resistance developed, the need for comprehensive and harmonized antimicrobial resistance surveillance programs was acknowledged on a global level. To that end, The World Health Organization encouraged countries to develop antimicrobial surveillance programs in the mid 1990's. These programs included surveillance of bacterial isolates originating from human, animal, and, where appropriate, retail meats with partnership among the following agencies: FDA (Center for Veterinary Medicine), CDC and USDA. The NARMS program began in 1996 and has been monitoring changes in antimicrobial drug susceptibilities of selected bacterial organisms in humans (CDC), animals (USDA, via VetNet, a molecular subtyping program, which was established in 2004), and retail meats (FDA). Collectively, NARMS and VetNet provide phenotypic and genotypic data on food borne pathogens and commensal bacteria. These data are critical for the production of wholesome meat and meat products and for USDA FSIS and CDC in achieving their public health missions. As part of the USDA-ARS appropriated program (#NP 108), this project is expected to continue supporting this national and international effort of monitoring antimicrobial resistance in food production animals. The provision for maintenance of well-characterized bacterial isolates supports current and future collaborative research.

Stated Objectives from original proposal

Objective 1: To elucidate and provide descriptive data, such as prevalence and/or trends, including antimicrobial susceptibilities, and molecular subtyping for food borne pathogens in food animals through the animal sampling arm of the NARMS program.

Objective 2: Be a national resource of enteric bacterial isolates and resistance data for food animals from NARMS and US-VetNet. This resource will facilitate the identification and characterization of antimicrobial resistance as it emerges. Further, it will facilitate the identification and implementation of any new research needs by the complementary research projects for various commodity groups.

Materials methods

Sample collections: A total of 4,426 fecal samples from 148 barns (1-2pens/barn) were collected (n=27-30/barn) from four states: NC, IA, OH and MN. The details are shown in Table 1. Fresh fecal samples were collected from each barn and shipped to USDA-ARS Bacterial Epidemiology and Antimicrobial Resistance (BEAR) laboratory in Athens, Georgia on the same day of collection for an overnight delivery. Sample collection schedule is as depicted on Table 1.

Isolation and identification of isolates: Each fecal sample was examined for the presence/absence of *Campylobacter, E. coli, Enterococcus, Salmonella* and MRSA at USDA-ARS, BEAR laboratory following standard methods routinely used in the laboratory.

Antimicrobial resistance testing: The isolates (*Campylobacter, E. coli, Salmonella, Enterococcus and MRSA*) were tested for antimicrobial resistance at USDA –ARS following CLSI M100 and NARMS protocols. The breakpoints and abbreviations of antimicrobials were used as described in the CLSI document.

State	Sept - Jan	Feb. - April	May - July	Aug - Sept	Total
North Carolina (A)	12	12	12	12	48
Iowa (B)	12	10	14	12	48
Ohio (C)	5	2	4	4	15
Minnesota (D)	6	10	10	11	37

Table 1: Fecal sample collections from four states (A, B, C and D)

Results

The prevalence of the major pathogens (*Campylobacter*, *E.coli and Salmonella*) from fecal samples collected from the four locations included in the study is shown in Table 2. The prevalence varied from 78%-89% (*Campylobacter*), 97.8%-99.5% (*E. coli*) and 3.3%-12.4% (*Salmonella*) among the four locations. There were six MRSA isolates identified in this study.

Prevalence of *Campylobacter, E. coli* and *Salmonella* by location					**MRSA**
State	***Campylobacter* (n=1184)**	***E. coli* (n=845)**	***Salmonella* (n=4,426*)***	***Enterococcus (n=?)***	**MRSA (n=?)**
North Carolina	85.2% (n=327)	97.8% (n=270)	12.4% (n=177)	80.4% (n=222)	0%
Iowa	85.9% (n=330)	98.6% (n=272)	9.3% (n=193)	70.3% (n=194)	0.4% (n=1)
Ohio	89.2% (n=107)	98.9% (n=89)	3.3% (n=82)	92.2% (n=83)	0%
Minnesota	77.7% (n=230)	99.5% (n=202)	12.3% (n=146)	74.4% (n=151)	2% (n=2)
Combined	83.6% (n=994)	98% (n=833)	10.4% (n=462)**	74% (n=650)	1% (n=3)

**yielded 481 isolates; 19 samples had 2 serotypes

Table 2: Prevalence of *Campylobacter, E coli* and *Salmonella* in fecal samples collected from pigs by location

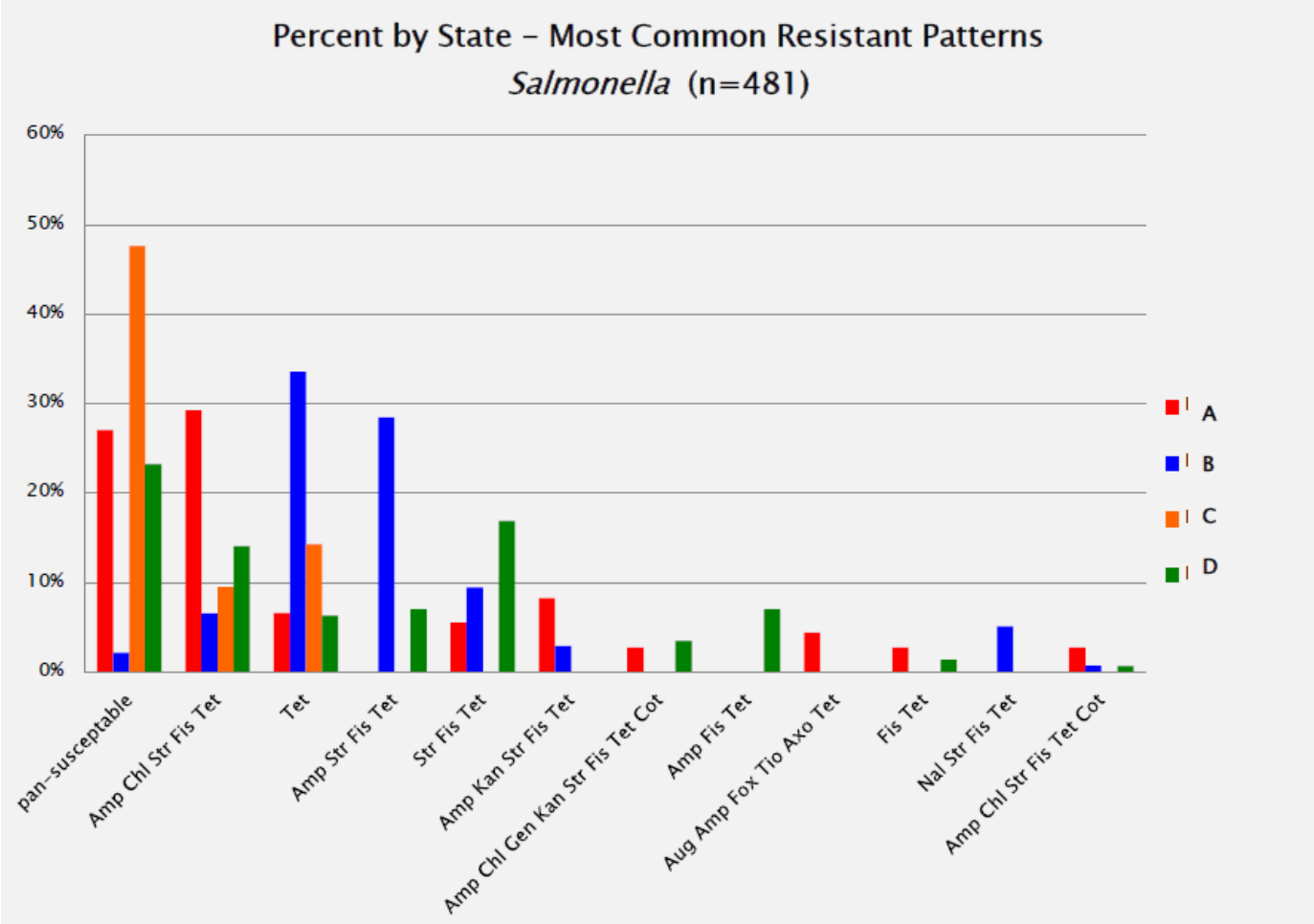
Among the 462 *Salmonella* isolates serotyped, different serotypes were identified of which *S.* Typhimuirium and S. Derby were predominant (Table 3).

Salmonella Serotype	**A (n=181)**	**B (n=137)**	**C (n=21)**	**D (n=142)**	**Total (n=481)**
Typhimurium, I 4, 5, 12 : i : -, or Albert*	89	57	9	46	201
Derby	15	71	1	33	120
Adelaide	0	0	2	23	25
Worthington	19	0	0	2	21
Infantis	11	0	1	5	17
Mbandaka	15	0	0	0	15
Alachua	0	0	0	11	11
Anatum	1	0	3	4	8
Rissen	8	0	0	0	8
Johannesburg	0	0	0	8	8

Table 3: Distribution of *Salmonella* serotypes identified from fecal samples by location

The proportion of antimicrobial resistant foodborne (*Salmonella* and *Campylobacter*) and commensal organisms (*E. coli* and *Enterococcus*) recovered from the study locations was relatively high and various antimicrobial resistance patterns (R-types) were detected (Table 4, 5 and 6). Figure 1 shows the most common resistance patterns detected among *Salmonella* serotypes (n=481). Resistance to tetracycline (76%), sulfisoxazole (59%), and streptomycin (55%) was higher compared to other antimicrobials included in the panel.

Antimicrobial resistance to *Campylobacter* species was frequently detected to tetracycline (87%), erythromycin (43%), azithromycin (43%), ciprofloxacin (14.8%) and gentamicin (0.8%). Among the *Campylobacter* isolates, *C. coli* was the predominant species (76%)/ recovered from pigs. Table 4 shows the resistance patterns (R-types) detected among *Campylobacter* isolates. The main R-types included Str Fis Tet (23%), Azm Cli Ery Tel Tet (16%) and Tet (38.5%).



The state codes include A =North Carolina, B =Iowa, C =Ohio and D =Minnesota.

Table 4: Antimicrobial resistance patterns among *Campylobacter spp (n=911)*

Resistance pattern (R-type)	Number of isolates	%
Azm Cip Cli Ery Nal Tel*	1	<1%
Azm Cip Cli Ery Nal Tel Tet	22	2.4%
Azm Cip Ery Nal Tet	7	<1%
Azm Cip Ery Gen Nal Tet	1	<1%
Azm Cip Ery Nal Tel Tet	12	1.3%
Azm Cip Ery Nal Tet	7	<1%
Azm Cli Ery	5	<1%
Azm Cli Ery Gen Tet	2	<1%
Azm Cli Ery Gen Nal Tet	1	<1%
Azm Cli Ery Tel	19	2.1%
Azm Cli Ery Tel Tet	141	15.5%
Azm Cli Ery Tet	32	3.5%
Azm Ery	6	<1%
Azm Ery Gen Tet	1	<1%
Azm Ery Nal Tel	3	<1%
Azm Ery Nal Tel Tet	1	<1%
Azm Ery Tel Tet	53	5.8%
Azm Ery Tel	3	<1%
Azm Tet	1	<1%
Cip Gen Nal Tet	1	<1%
Cip Nal	7	<1%
Azm Ery Tet	73	8%
Cip Nal Tet	76	<1%
Cli Tet	1	<1%
Gen Tet	4	<1%
Tet	351	38.5%
Pansusceptible	78	8.6%

*Ami= Amikacin; Amp= Ampicillin; Apr=Apramycin; Azm= Azithromycin; Gen= Gentamicin; Kan= Kanamycin; Lin=Clindamycin; Cep= Cephalothin; Tio= Ceftiofur; Str =Streptomycin; Aug= Amoxicillin-Clavulanic Acid; Tic= Ticarcillin; Fox= Cefoxtin; Axo= Ceftriaxone; Smx/Fis= Sulphonamides, Sulfamethoxazole/Sulfisoxzole; Azi= Azithromycin; Chl= Chloramphenicol; Cip= Ciprofloxacin; Nal= Nalidixic acid; Tel= Telithromycin; Tet= Tetracycline

Among *E. coli* isolates (n=833), antimicrobial resistance was detected to tetracycline (89%), sulfonamides (33%) and resistance to third generation cephalosporins (<6%) and to ciprofloxacin (1.7%). Different R-types were detected among *E. coli* isolates (Table 5) and included Str Fis Tet (23%) and Tet (33%).

Table 5: Antimicrobial resistance patterns among *Escherichia coli isolates* (n=830)

Resistance pattern (R-type)	**Number of isolates**	**% (Proportion)**
Amp	2	<1%
Amp Azm Chl Kan Str Fis Tet Cot	1	<1%
Amp Chl Cip Gen Kan Nal Str Fis Tet Cot	1	<1%
Amp Chl Fis Tet	8	<1%
Amp Chl Fis Tet Cot	6	<1%
Amp Chl Gen Str Fis Tet	1	<1%
Amp Chl Gen Str Fis Tet	3	<1%
Amp Chl Kan Str Fis Tet	1	<1%
Amp Chl Kan Str Tet	1	<1%
Amp Chl Str Fis	1	<1%
Amp Chl Fis Tet	3	<1%
Amp Chl Str Fis Tet Cot	1	<1%
Amp Cip Kan Nal Fis Tet Cot	1	<1%
Amp Fis Tet	13	1.6%
Amp Fis Tet Cot	7	<1%
Amp Gen Fis Tet	2	<1%
Amp Gen Kan Str Fis Tet	2	<1%
Amp Gen Kan Tet	1	<1%
Amp Gen Str Fis Cot	1	<1%
Amp Gen Tet	2	<1%
Amp Kan Fis Tet	6	<1%
Amp Kan Tet	3	<1%
Amp Nal Str Fis Tet Cot	1	<1%
Amp Kan Str Tet	6	<1%
Amp Str Fis Tet	10	1.2%
Amp Str Tet	32	3.8%
Amp Tet	54	6.5%
Amp Tio Axo Chl Fis Tet	1	<1%
Amp Tio Axo Chl Fis Tet Cot	1	<1%
Aug Amp Axo Gen Kan Str Fis Tet Cot	1	<1%
Aug Amp Fox Axo	1	<1%
Aug Amp Fox Axo Chl Str Fis Tet	1	<1%
Aug Amp Fox Axo Chl Str Fis	1	<1%
Aug Amp Fox Tio Axo	2	<1%
Aug Amp Fox Tet	1	<1%
Aug Amp Fox Tio Axo Chl Gen Kan Str Fis Tet Cot	2	<1%
Aug Amp Fox Tio Axo Chl Cip Nal Str Fis Tet	1	<1%
Aug Amp Fox Tio Axo Chl Gen Fis Tet Cot	1	<1%
Aug Amp Fox Tio Axo Chl Gen Str Fis Tet Cot	1	<1%
Aug Amp Fox Tio Axo Chl Kan Str Fis Tet	3	<1%

Aug Amp Fox Tio Axo Chl Gen Fis Tet	2	<1%
Aug Amp Fox Tio Axo Chl Gen Fis Tet Cot	1	<1%
Aug Amp Fox Tio Axo Chl Tet	2	<1%
Aug Amp Fox Tio Axo Cip Kan Nal Str Fis Tet Cot	1	<1%
Aug Amp Fox Tio Axo Cip Nal Fis Tet Cot	1	<1%
Aug Amp Fox Tio Axo Fis Tet	3	<1%
Aug Amp Fox Tio Axo Gen Fis Tet Cot	1	<1%
Aug Amp Fox Tio Axo Gen Str Fis Tet Cot	1	<1%
Aug Amp Fox Tio Axo Gen Kan Fis Tet	2	<1%
Aug Amp Fox Tio Axo Kan Fis Tet	1	<1%
Aug Amp Fox Tio Axo Kan Nal Str Fis Tet	1	<1%
Aug Amp Fox Tio Axo Kan Str Fis	1	<1%
Aug Amp Fox Tio Axo Nal Tet	1	<1%
Aug Amp Fox Tio Axo Str Tet	3	<1%
Aug Amp Fox Tio Axo Tet	3	<1%
Aug Amp Gen Fis Tet	1	<1%
Azm Gen Fis Tet Cot	1	<1%
Chl Cip Kan Nal Fis Tet Cot	2	<1%
Chl Cip Nal Fis Cot	1	<1%
Chl Fis	1	<1%
Chl Fis Tet	25	3%
Chl Fis Tet Cot	2	<1%
Chl Gen Fis	1	<1%
Chl Kan Str Fis Tet	6	<1%
Chl Kan Fis Tet	3	<1%
Chl Str Fis	1	<1%
Chl Str Fis Tet	3	<1%
Chl Str Tet	1	<1%
Chl Tet	7	<1%
Cip Gen Nal Fis Tet Cot	1	<1%
Cip Nal Tet	5	<1%
Fis Cot	1	<1%
Fis	1	<1%
Fis Tet	15	1.8%
Fis Tet Cot	11	1.3%
Gen Kan Str Fis Tet	1	<1%
Gen Nal Str Fis Tet	1	<1%
Gen Str Fis Tet	1	<1%
Gen Str Tet	2	<1%
Kan	4	<1%
Kan Fis Tet	11	1.3%
Kan Fis Tet Cot	2	<1%
Kan Str Fis Tet	36	4.3

Kan Str Fis Tet Cot	1	<1%
Kan Str Tet	8	2.7%
Kan Tet	10	3.3%
Nal Tet	3	<1%
Str	2	<1%
Str Fis	2	<1%
Str Fis Tet	19	22.9%
Str Fis Tet Cot	1	<1%
Str Tet	3	<1%
Tet	276	32.5%
Pansusceptible	67	8.1%

The majority of *Enterococcus* isolates belonged to *Ent. fecalis* (46%; 126/276)) followed by *Ent. hirae* (36%; 98/276). About 77% (212/276) of the *Enterococcus* isolates were resistant to three or more antimicrobials (MDR). The main resistance patterns observed among *Enterococcus* isolates included resistance to EryLinSynTetTyl (23%) and to LinSynTet (23%), Table 6.

Table 6: Antimicrobial resistance patterns among *Enterococcus* isolates (n=276)

Resistance pattern (R-type)	**Number of isolates**	**% (Proportion)**
Chl Cip Ery Gen Kan Lin Syn Tet Tyl	1	<1%
Chl Cip Ery Gen Kan Lin Syn Tet Tyl	1	<1%
Chl Ery Kan Lin Str Syn Tet Tyl	5	1.8%
Chl Ery Lin Syn Tet Tyl	7	2.5%
Cip Ery Kan Lin Nit Pen Str Syn Tet Tyl	2	<1%
Cip Lin Nit Pen Str Tet	1	<1%
Cip Lin Nit Syn Tet	2	<1%
Cip Lin Tet	1	<1%
Cip Nit	1	<1%
Dap Ery Lin Syn Tet Tyl	3	<1%
Dap Lin	1	<1%
Dap Lin Nit	1	<1%
Dap Lin Str Syn Tet	1	<1%
Dap Lin Str Tet	1	<1%
Dap Lin Tet	6	2.2%
Dap Lin Syn	1	<1%
Ery Gen Kan Lin Str Syn Tet Tyl	6	2.2%
Ery Gen Kan Lin Str Tet Tyl	1	<1%
Ery Gen Kan Lin Syn Tet Tyl	5	1.8%
Ery Kan Lin Nit Str Syn Tet Tyl	1	<1%
Ery Kan Lin Str Syn Tet Tyl	13	4.7%
Ery Kan Lin Str Tet Tyl	1	<1%

Ery Kan Lin Syn Tet Tyl	3	1.1%
Ery Lin Nit Str Syn Tet Tyl	1	<1%
Ery Lin Nit Syn Tet Tyl	5	1.8%
Ery Lin Nit Tet Tyl	1	<1%
Ery Lin Pen Str Tet Tyl	1	<1%
Ery Lin Str Syn Tet Tyl	12	4.3%
Ery Lin Syn Tet Tgc Tyl	1	<1%
Ery Lin Syn Tet Tyl	59	21.4%
Ery Lin Tet Tyl	5	1.8%
Kan Lin Syn Tet	1	<1%
Lin	5	1.8%
Lin Nit	3	1.1%
Lin Nit Pen Str Syn Tet	1	<1%
Lin Nit Pen Syn Tet	1	<1%
Lin Nit Str Tet	1	<1%
Lin Nit Syn Tet	1	<1%
Lin Nit Tet	6	2.1%
Lin Str Syn Tet	4	1.4%
Lin Pen Tet	1	<1%
Lin Str Tet	2	<1%
Lin Syn	6	2.1%
Lin Syn Tet	45	16.3%
Lin Tet	46	16.7%
Lin Tet Tgc	1	<1%
Nit	1	<1%
Nit Tet	1	<1%

Of the total fecal samples examined for MRSA, three positive samples were detected and showed resistance to AmpOxaPenTet (n=2) and Amp Pen Tet (n=1).

Objective 2: Be a national resource of enteric bacterial isolates and resistance data for food animals from NARMS and US-VetNet.

The collected and characterized isolates within the one year period alone include: Campylobacter (n=1184); E. coli (n=845) and Salmonella (n=4,426). This pilot project, from four locations representing more than 51% of the pig population, can serve as a spring board in facilitating the identification and characterization of emerging antimicrobial resistance particularly among enteric bacteria which are of significance to the pork producers and public health. This is an important resource for further investigation to conduct a targeted hypothesis-driven research on the various foodborne and indicator organisms representing the swine industry.

Discussion

Results of the pilot study indicated that antimicrobial resistant foodborne (Salmonella and Campylobacter) and commensals (E. coli and Enterococcus) are prevalent in the study locations and multidrug resistance was commonly detected in both foodborne (*Salmonella* and *Campylobacter*) and commensal (*E. coli* and *Enterococcus*) organisms recovered from three major pig producing geographic locations in the US as well as from Ohio. The findings also suggest the need for continuous and comprehensive monitoring of antimicrobial resistance programs in the swine industry. The key limitation of this on-farm monitoring project particularly that affected objective #2 is the lack of funding and federal coordination to continue this monitoring project. While the more than 6500 isolates can be resources for further hypothesis-driven research, the fact that it is collected within only one year period is makes it difficult to conduct longitudinal studies.