

Prevalence and Antimicrobial Resistance of *Salmonella*, *E. coli*, and *Campylobacter* in Pigs from Swine Producing States in the United States

Abley, M.⁽¹⁾, Fedorka-Cray, P.J.^{(1)*}, Gebreyes, W.⁽²⁾, McKean, J.⁽³⁾, Davies, P.⁽⁴⁾, Thakur, S.⁽⁵⁾, Larsen, S.⁽⁶⁾

⁽¹⁾ USDA-ARS, BEAR, 950 College Station Rd., Athens, GA 30605

⁽²⁾ The Ohio State University, Columbus, OH

⁽³⁾ Iowa State University, Ames, IA

⁽⁴⁾ University of Minnesota, St. Paul, MN

⁽⁵⁾ North Carolina State University, Raleigh, NC

⁽⁶⁾ National Pork Board, Des Moines, IA

*corresponding author: paula.cray@ars.usda.gov

Abstract

The purpose of the study was to determine the prevalence and antimicrobial susceptibility of *Salmonella*, *Campylobacter* and generic *E. coli* (commensal bacteria which may harbor antimicrobial resistance genes) from swine feces collected over one year from the top three swine producing states (Iowa, North Carolina, and Minnesota), which represent 51% of the total pig crop in the U.S, plus Ohio. The prevalence of *Salmonella* (n=462/4426), *Campylobacter* (n=994/1184) and *E. coli* (n=833/845) at the sample level was 10.4%, 98.6% and 83.6%, respectively. Overall, the top three *Salmonella* serotypes were Typhimurium (42%), Derby (25%) and Adelaide (5%) while *C. coli* was the predominant *Campylobacter* species. *Salmonella* serotypes varied by barn within state and strain differences within serotypes by antibiogram and pulsotype were observed. In general, *Salmonella* were most often resistant to Tetracycline (76%), Sulfisoxazole (59%), and Streptomycin (55%); however, serotype variation occurred. *E. coli* was most often resistant to Tetracycline (89%) and the Sulfonamides (33%); *C. coli* were most often resistant to Tetracycline (87%), Erythromycin (43%) and Azithromycin (43%). Less than 5.8% of *E. coli* and 5.6% of *Salmonella* were resistant to Ceftriaxone, a clinically important antimicrobial. Seasonal variations by serotype and resistance were observed.

Introduction

Foodborne illness is a global problem and foodborne infections with *Salmonella* and *Campylobacter* continue to be problematic in the United States with 1.03 million and 0.8 million, respectively episodes of foodborne illnesses a year (1). Collectively, foodborne illness costs in the U.S. are projected to be \$152 billion per year (2). Although gastroenteritis associated with foodborne infections often resolves without treatment, the development of antimicrobial resistance to clinically important antimicrobials remains a significant concern when treatment is indicated (3). Antimicrobials are important for use in both human and food animal production.

Studies have been conducted to determine the prevalence and antimicrobial resistance of food borne pathogens in swine (4,5,6). The purpose of the study was to determine the prevalence and antimicrobial susceptibility of *Salmonella*, *Campylobacter* and generic *E. coli* (commensal bacteria which may harbor antimicrobial resistance genes) from swine feces collected over one year from the top three swine producing states (Iowa, North Carolina, and Minnesota), which represent 51% of the total pig crop in the U.S, plus Ohio.

Material and Methods

Using recent projections from USDA NASS (7), operations with more than 5,000 head accounted for 61.1% of the total hog and pig inventory (projected at approximately 68 million pigs) in 2008 with the ten largest states (Iowa, North Carolina, Minnesota, Illinois, Indiana, Nebraska, Missouri, Oklahoma, Ohio, and Kansas) accounting for over 85% of this inventory. Therefore, we started initial sampling in the top three states, Iowa, North Carolina, and Minnesota which represented 51% of the total swine inventory. Based on National Animal Health Monitoring System Swine 2006 projections of 7.2% *Salmonella* prevalence on-farm (8), the expected total number of *Salmonella* isolates recovered were projected to equal approximately 324 which exceeded our target number of 300 *Salmonella* isolates.

Up to 30 fresh fecal samples were collected per barn pen floor from a total of 148 barns across all states (n=4,426 samples collected from a majority of 1-2 pens/barn) (Table 1); collections were divided by season. Ohio was included to represent smaller operations and contributed 3 barns per quarter (12 barns total).

Salmonella, *E. coli* and *Campylobacter* were isolated using standard culture methods. Antimicrobial susceptibility testing was determined using broth microdilution (Sensititer, Trek Diagnostic System, ThermoFisher Scientific Inc., Cleveland, OH)

and a custom panel of 15 antimicrobial agents according to manufacturer's directions. Resistance was determined using CLSI interpretive standards (9,10).

Molecular subtyping was determined using pulsed field gel electrophoresis (11).

Results

The prevalence of *Salmonella* (n=462/4426), *Campylobacter* (n=994/1184) and *E. coli* (n=833/845) at the sample level was 10.4%, 98.6% and 83.6%, respectively. Overall, the top three *Salmonella* serotypes were Typhimurium (42%), Derby (25%) and Adelaide (5%). Serotypes by state are shown in Table 2.

Salmonella serotypes varied by barn within state and strain differences within serotypes by antibiogram and pulsotype were observed. In general, *Salmonella* were most often resistant to Tetracycline (76%), Sulfisoxazole (59%), and Streptomycin (55%); however, serotype variation occurred. Seasonal variations by serotype and resistance were observed.

C. coli was the predominant *Campylobacter* species which were most often resistant to Tetracycline (87%), Erythromycin (43%) and Azithromycin (43%). *E. coli* was most often resistant to Tetracycline (89%) and the Sulfonamides (33%). Less than 5.8% of *E. coli* and 5.6% of *Salmonella* were resistant to Ceftriaxone, a clinically important antimicrobial.

Discussion

The prevalence of *Salmonella* was 10.4% based on a recovery of 462 positive samples from 4,426 submitted samples. Of the 462 positive samples, 2 serotypes were recovered from 19 samples yielding 481 isolates. It is interesting to note the distribution of serotypes across states in Table 2. With the exception of the top two serotypes and Infantis and Anatum which are recovered from three states, no other serotype is recovered in more than two states.

It is also interesting to note that there is considerable within serotype diversity with respect to within barn recovery by state (data not shown). This may be attributed to the movement of pigs themselves which carry different bacterial flora or the transmission of bacteria by other means. Further diversity within serotype is also noted with respect to acquisition of resistance as some phenotypes exhibit more resistance (Typhimurium) while others tend to exhibit little or no resistance or resistance primarily to the historical antimicrobials (streptomycin, sulfas, tetracycline) (Derby) (data not shown). Although seasonal variation in both prevalence and resistance would not be unexpected, the effect on serotypes and subsequent impact on resistance should be monitored.

Campylobacter coli is not typically associated with human illness. However, since Erythromycin is a drug of choice in human medicine continued surveillance is warranted in addition to the emerging resistance to

Table 1. Number of barns sampled per site.

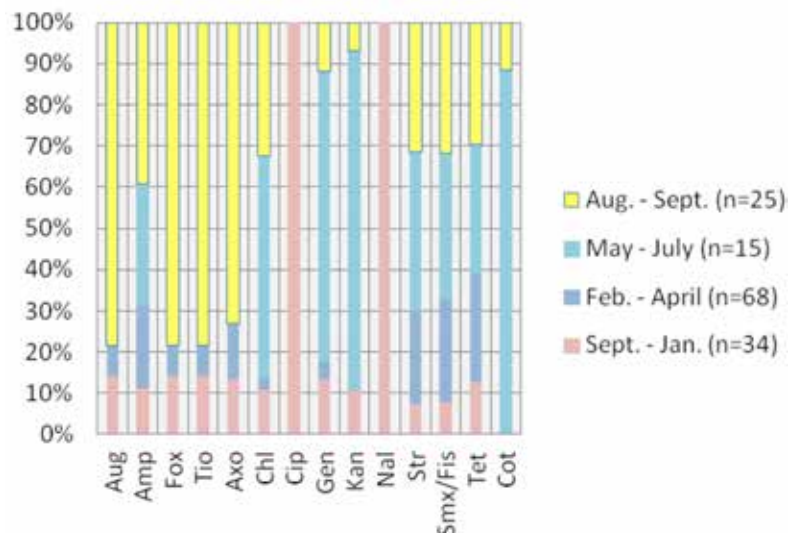
	Sept. - Jan.	Feb. - April	May - July	Aug. - Sept.	Total
A	12	12	12	12	48
B	12	10	14	12	48
C	5	2	4	4	15
D	6	10	10	11	37

Table 2. *Salmonella* serotypes by state

Serotypes	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

*serotyping was done by using the SMART molecular PCR developed in our laboratory which does not differentiate between Typhimurium, I 4,5,12 : i : -, or Albert ; Rissen was confirmed by PFGE

Figure 1. Seasonal variation in antimicrobial resistance in all *Salmonella* spp. from state D (n=142)



Ceftriaxone, the quinolone Nalidixic Acid, and fluoroquinolone Ciprofloxacin in *E. coli* and *Salmonella*.

Conclusion

Further characterization of persistent versus non-persistent bacterial strains including those that readily acquire resistance more than others may offer areas for development of mitigation strategies.

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