

PREVALENCE AND ANTIMICROBIAL RESISTANCE OF *SALMONELLA* ISOLATED FROM CONVENTIONAL AND ANTIMICROBIAL-FREE (ABF) SWINE HERDS IN NORTH CAROLINA

Wondwossen A. Gebreyes^{1*}, Siddhartha Thakur¹, W.E. Morgan Morrow²

¹North Carolina State University, College of Veterinary Medicine, Dept of Population Health and Pathobiology, 4700 Hillsborough ST., Raleigh, NC 27606, USA, Ph: 01-919-513-8291, E-mail: wagebrey@ncsu.edu; ²College of Agriculture and Life Sciences, Dept. of Animal Sciences

Summary The main objective of this study was to compare prevalence and antimicrobial resistance of *Salmonella* isolated from swine reared in antimicrobial-free (ABF) and conventional production systems in North Carolina. Among the two production systems, prevalence was significantly higher among the ABF (15%) than the conventional system (5.2%) ($P < 0.001$). There was no significant difference in the resistance against tetracycline between the two production system at the farm level ($P = 0.22$). The AMP CHL STR SUL TET (R-type ACSSuT) MDR pattern was the most common seen in 73 (10.4%) of the isolates. Among the two production systems, this penta-resistance pattern was observed significantly higher in the ABF (20.6%) than the conventional (2.4%) herds at the farm level ($P < 0.001$). The common finding of highly MDR resistant strains in ABF farms may indicate that specific MDR strains may remain persistent in swine production units regardless of antimicrobial use levels.

Introduction Non-typhoidal *Salmonella* serovars have been known to be among the most common bacterial pathogens and important reservoirs of antimicrobial resistance. *Salmonella* has been commonly identified from commercial swine farms in North Carolina (Davies *et al.*, Funk *et al.*, Gebreyes *et al.*). Antimicrobial resistant strains of the most common serovars including Typhimurium and Muenchen has been reported recently in pigs (Gebreyes and Altier, 2002; Gebreyes *et al.*, 2004; Gebreyes *et al.*, 2004B; Gebreyes and Thakur, 2005). The previous studies have mainly focused on the conventional production systems. In the current study, we compared the prevalence and antimicrobial resistance of *Salmonella* isolated from swine reared in antimicrobial-free (ABF) and conventional production systems in North Carolina.

Materials and Methods Fecal samples from a total of 889 pigs and 743 carcass swabs were collected from 21 groups of pigs reared in conventional (11 groups) and ABF (10 groups) farms in a cross-sectional study. Under the conventional system of pig production, antimicrobials were added in the feed for growth promotion and also used for therapeutic purposes. The ABF production system is different with no antimicrobials used for any purpose post-weaning. Approximately 10 grams of fresh fecal matter was collected from each pig per rectum with sterile gloves. At the slaughter plant, we sampled carcasses using swabs soaked in 10 ml of buffered peptone water (Becton Dickinson, NJ, USA). The region extending from the jowl to the ham was swabbed in a single swipe. Ten carcasses each were swabbed at three different points (pre-evisceration, post-evisceration and post-chill) at the slaughter plant making it a total of 30 carcasses sampled per trip. All the 30 carcasses were swabbed at the plant. The samples were transported to the laboratory on ice for isolation of salmonellae.

Salmonella isolation from fecal samples was done following the conventional methods described previously (Gebreyes *et al.*, 2004). After, culturing on selective plates, five colonies characteristic of *Salmonella* were further tested for the appropriate biochemical reactions on triple sugar iron (TSI) and urea agar media (Difco Ltd., Sparks, MD). The isolates were tested for their antimicrobial susceptibility to 12 antimicrobial agents using the Kirby-Bauer disk diffusion method. The antimicrobials tested, abbreviations and disk potency used were: ampicillin (AMP) [10mg], amoxicillin-clavulanic acid (AMX) [30mg], amikacin (AMK) [30mg], ceftriaxone (CRO) [30mg], cephalothin (CEF) [30mg], chloramphenicol (CHL) [30mg], ciprofloxacin (CIP) [5 mg], gentamicin (GEN) [10mg], kanamycin (KAN) [30mg], streptomycin (STR) [10mg], sulfamethoxazole (SUL) [250mg] and tetracycline (TET) [30mg]. Results were interpreted according to the NCCLS criteria. *Escherichia coli* strain ATCC 25922 and 35218, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853 were routinely

used as quality control organisms according to NCCLS recommendations.

To compare the prevalence, antimicrobial resistance profile and patterns of *Salmonella* isolates between the two production systems, we used the χ^2 test (Minitab Inc. PA, USA) and Fisher's exact two-tailed test (www.matforsk.no/ola/fisher.htm) wherever applicable. An α of 0.05 was used as significance level.

Results *Salmonella* prevalence at farm was 9.3% (n=83) and 10.6% (n=79) at the slaughter as shown in Figure 1. Among the two production systems, prevalence was significantly higher among the ABF (15%) than the conventional system (5.2%) ($P<0.001$). Overall, we did not find any significant difference in prevalence between the farm and slaughter ($P=0.33$). Within the conventional production units, although prevalence was higher at slaughter (6.8%) than the farm (4.2%), the difference was not significant ($P=0.09$). We observed similar result within the ABF system with no significant difference between the farm and slaughter ($P=0.9$).

Resistance was detected against 10 of the 12 antimicrobials tested either at farm or slaughter except ciprofloxacin and amikacin (Table 1). Overall, frequency of resistance against tetracycline was the highest (80% isolates) followed by streptomycin (43.4%) and sulfamethoxazole (36%) irrespective of the production system or the production stage. There was no significant difference in the resistance against tetracycline between the two production system at the farm level ($P=0.22$). However, we found significant difference ($P<0.001$) in resistance against this antimicrobial at the slaughter level with higher resistance seen among isolates from the conventional system (97.4%) than the ABF system (55.7%). Frequency of resistance against ampicillin, streptomycin and sulfamethoxazole was higher in *Salmonella* isolates from pigs reared in the conventional system at both farm and slaughter ($P<0.001$). *Salmonella* strains resistant to the third generation cephalosporin ceftriaxone (n=2) and gentamicin (n=2) were isolated at the slaughter and farm level respectively among the conventionally reared pigs. None of the isolates from ABF slaughter level were resistant to chloramphenicol and kanamycin.

A total of 28 resistance patterns were observed among the total 703 *Salmonella* isolates at farm and slaughter. A total of 116 isolates (16.5%) were pansusceptible. At the farm level, a single isolate (1.2%) from the conventional production system was pansusceptible compared to 33 (11.7%) of the isolates from the ABF farms. Pansusceptible *Salmonella* isolates at the slaughter level were found in carcasses from the ABF production system (82; 37%) but none from carcasses of the conventional herds. The resistance pattern STR SUL TET was the most common pattern and seen in 130 (18.5%) of the 703 isolates. There was no significant difference in the proportion of isolates with the above pattern between the conventional (19.5%) and the ABF system (18%) ($P=0.66$). We observed a total of 21 multidrug resistance (MDR) patterns exhibited by 147 (21%) isolates. MDR here is defined as isolates exhibiting resistance to four or more antimicrobials simultaneously. The AMP CHL STR SUL TET MDR pattern was the most common seen in 73 (10.4%) of the isolates. Among the two production systems, this pentaresistance pattern was observed significantly higher in the ABF (20.6%) than the conventional (2.4%) at the farm level ($P<0.001$). None of the isolates from the ABF slaughter carcasses exhibited this MDR pattern. Isolates with the MDR pattern STR SUL TET KAN (17.4%) were isolated only from the carcasses of conventionally reared pigs. The other resistance patterns that were seen in isolates from the slaughter plants only included AMP STR AMX CEF (1.2%), AMP STR TET AMX CEF (0.9%), AMP TET AMX CEF CRO (1.8%), AMP CHL STR SUL TET AMX CEF (0.9%) and STR SUL TET CEF (1.2%).

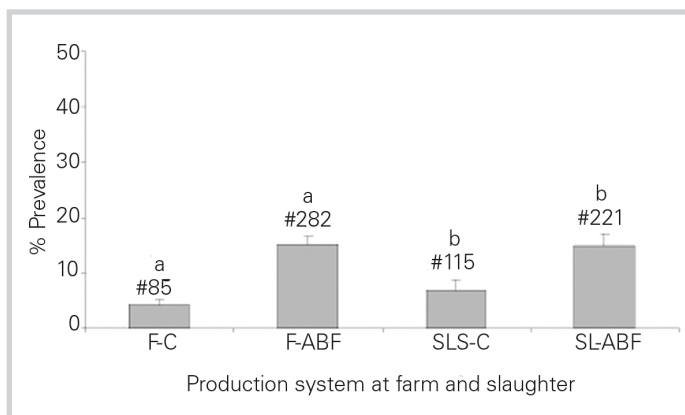


Figure 1. *Salmonella* prevalence at farm and slaughter among the conventional and the ABF production systems. Abbreviations: F-C (Conventional Farms); F-ABF (ABF Farms); SLS-C (Conventional Slaughter); SL-ABF (ABF Slaughter) *^a Bars sharing common superscripts were significantly different at $P<0.05$; # Indicates number of pigs/carcass sampled

Discussion In this study, comparison of prevalence and antimicrobial resistance of *Salmonella* has been conducted in conventional and antimicrobial-free swine production systems in North Carolina. Important findings on differences in prevalence and antimicrobial resistance between ABF and conventional herds have been identified. One of the initial findings is the high prevalence of *Salmonella* in ABF herds than conventional ones and has been consistent both on-farm and at slaughter as shown on Figure 1. Previously, it has been reported that in production systems where antimicrobials were not used, a higher prevalence of other swine specific infectious agents (ileitis due to *Lawsonia intracellularis*) and clinical illnesses have been reported from other countries (Ref.). Our findings could corroborate such reports and may imply that banning of antimicrobial use in swine production may lead to an increase in prevalence of *Salmonella*.

The other major finding of this report is the high occurrence of antimicrobial resistance in both ABF and conventional production systems. Resistance to tetracycline, the antimicrobial to which *Salmonella* strains have widely been known to be commonly resistant, was shown to be common in both ABF and conventional herds. However, frequency of resistance to other antimicrobials including β -lactams, aminoglycosides and sulfamethoxazole was more common among isolates from conventional herds than ABF. On further investigating the specific MDR patterns, the pan-demically recognized pentaresistance pattern commonly known as R-type ACSSuT was detected significantly more commonly from the ABF herds on-farm than the conventional. Even though we do not know the specific risk factors that may have resulted in such a high occurrence of this pentaresistance pattern in ABF herds, it could also be argued that it may be attributed to factors other than antimicrobial use. It may also imply the fact that MDR strains may remain persistent in production and other environments regardless of antimicrobial use levels. Previously, it has been shown that secondary mutations in the genome of such MDR strains may render them to be fit to survive in various conditions (Jorkman *et al.*, 2000).

Conclusions The high occurrence of *Salmonella* in the ABF herds may be associated with the antimicrobial use status of the herds. As this study was done based on convenience sampling and on limited farms, it may not have external validity and thus can not be generalized. The findings, however, warrant the need for a more comprehensive study. The common finding of highly MDR resistant strains in ABF farms also may indicate that specific MDR strains may remain persistent in production and other environments regardless of antimicrobial use levels.

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Production Stage	Production System	Isolates Tested	Antimicrobials											
			AMP	CHL	STR	SUL	TET	AMX	CEF	CRO	CIP	KAN	AMK	GEN
Farm	Conventional	85	30 (35.3) ¹	22 (25.9) ²	73 (85.9) ³	55 (64.7) ⁴	79 (93)	12 (14.1)	5 (5.9)	0	0	16 (18.8)	0	2 (2.4)
	ABF ^a	282	35 (12.4)	30 (10.6)	102 (36.2)	85 (30.1)	249 (88.3)	2 (0.7)	1 (0.4)	0	0	4 (1.4)	0	0
Slaughter	Conventional	115	27 (23.5) ¹	25 (21.7) ²	63 (54.8) ³	58 (50.4) ⁴	112 (97.4)	4 (3.5)	3 (2.6)	2 (1.7)	0	20 (17.4)	0	0
	ABF	221	7 (3.2)	0	67 (30.3)	56 (25.3)	123 (55.7)	4 (1.8)	5 (2.3)	0	0	0	0	0
Total Isolates		703	99 (14)	77 (11)	305 (43.4)	254 (36.1)	563 (80)	22 (3.1)	14 (2)	2 (0.3)	0	40 (5.7)	0	2 (0.3)

Table 1. Comparison of antimicrobial resistance profile between *Salmonella* isolates from the two production system. ^aAntimicrobial Free Farms. For each antimicrobial, figures sharing common digits in the superscripts were significantly different at $P < 0.05$