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Comparison of Prevalence, Antimicrobial Resistance, and Occurrence of Multidrug-Resistant *Salmonella* in Antimicrobial-Free and Conventional Pig Production

WONDWOSSEN A. GEBREYES,[1]* SIDDHARTHA THAKUR,[1] AND W. E. MORGAN MORROW[2]

[1]*College of Veterinary Medicine, Department of Population Health and Pathobiology and* [2]*College of Agriculture and Life Sciences, Department of Animal Sciences, North Carolina State University*

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ABSTRACT

Conventional swine production evolved to routinely use antimicrobials, and common occurrence of antimicrobial-resistant *Salmonella* has been reported. There is a paucity of information on the antimicrobial resistance of *Salmonella* in swine production in the absence of antimicrobial selective pressure. Therefore, we compared the prevalence and antimicrobial resistance of *Salmonella* isolated from antimicrobial-free and conventional production systems. A total of 889 pigs and 743 carcasses were sampled in the study. *Salmonella* prevalence was significantly higher among the antimicrobial-free systems (15.2%) than the conventional systems (4.2%) (odds ratio [OR] = 4.23; $P < 0.05$). Antimicrobial resistance was detected against ten of the twelve antimicrobials tested. The highest frequency of resistance was found against tetracycline (80%), followed by streptomycin (43.4%) and sulfamethoxazole (36%). Frequency of resistance to most classes of antimicrobials (except tetracycline) was significantly higher among conventional farms than antimicrobial-free farms, with ORs ranging from 2.84 for chloramphenicol to 23.22 for kanamycin at the on-farm level. A total of 28 antimicrobial resistance patterns were detected. A resistance pattern with streptomycin, sulfamethoxazole, and tetracycline ($n = 130$) was the most common multidrug resistance pattern. There was no significant difference in the proportion of isolates with this pattern between the conventional (19.5%) and the antimicrobial-free systems (18%) (OR = 1.8; $P > 0.05$). A pentaresistance pattern with ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline was strongly associated with antimicrobial-free groups (OR = 5.4; $P = 0.01$). While showing the higher likelihood of finding antimicrobial resistance among conventional herds, this study also implies that specific multidrug-resistant strains may occur on antimicrobial-free farms. A longitudinal study with a representative sample size is needed to reach more conclusive results of the associations detected in this study.

Nontyphoidal *Salmonella* serovars have been known to be among the most common bacterial foodborne pathogens worldwide and important reservoirs of antimicrobial resistance. An intensive food animal production system, which includes conventional commercial swine production, has evolved to use antimicrobials routinely as a prophylactic measure to prevent the spread of infectious agents as well as for growth promotion purposes in the last half a century. Such routine antimicrobial use for production purposes has been implicated as an important selective pressure for emergence and dissemination of antimicrobial-resistant bacterial strains *(22).* Non–host-adapted, ubiquitous nontyphoidal *Salmonella* serovars are among the common foodborne agents known to act as reservoirs of antimicrobial resistance. Multidrug-resistant *Salmonella* from humans and various food and companion animals has been a worldwide problem in the last two decades *(3, 20, 23). Salmonella* serovars are commonly identified from commercial swine farms in the United States *(2, 5, 7, 9, 14).* Antimicrobial-resistant strains, particularly the most common serovars such as Typhimurium and others, have also been commonly reported *(6, 8, 10, 11, 21).* The wide occurrence of such resistant strains has been documented in different pathogens and geographic areas *(16).* Studies to date mainly targeted conventional production systems where antimicrobials are routinely used for treatment and growth promotion purposes. There is little information on the antimicrobial resistance levels of *Salmonella* in swine production units in the absence of antimicrobial use selective pressure. The role of other known or unidentified selective pressures that may result in persistence of resistant strains remains to be investigated. In the current study, we compared the prevalence and antimicrobial resistance of *Salmonella* isolated from antimicrobial-free and conventional production systems in North Carolina. This study is among the first comparing the two swine production systems in United States. In addition, occurrence of multidrug-resistant strains and phenotypic diversity between the two production types and between production and processing stages were investigated.

MATERIALS AND METHODS

Sample collection. Fecal samples and carcass swabs were collected at farm and slaughter as part of a cross-sectional study involving 20 groups of pigs reared in conventional (10 herds) and antimicrobial-free (ABF; 10 herds) production systems in North

* Author for correspondence. Tel: 919-513-8291; Fax: 919-515-3044; E-mail: wagebrey@ncsu.edu.

Carolina sampled between October 2002 and October 2004. All the conventional farms were located in two counties in the southeastern part of the state. The ABF farms were located in the northeastern ($n = 5$) and southeastern ($n = 5$) parts of the state. The minimum distance between the two types of production systems was estimated to be 20 miles. Under the conventional system of pig production, antimicrobials were added to the feed for growth promotion and were also used for therapeutic purpose. No antimicrobials were used for any purpose in the ABF production postweaning. Sick pigs that were given antimicrobials for treatment in the ABF units were immediately removed from the herd, kept in a different barn or pen, and marketed as conventional. They were excluded from this study. Overall, a total of 889 individual pig fecal samples (475 from conventional and 414 from ABF) and 743 carcass swabs (381 from conventional and 362 from ABF) were sampled at farm and slaughter, respectively. Approximately 10 g of fresh fecal matter was collected from each pig per rectum with sterile gloves. At the slaughter plant, we sampled carcasses with swabs soaked in 10 ml of buffered peptone water (BPW; Becton Dickenson, Franklin Lakes, N.J.). The samples were transported on ice to the laboratory for isolation of salmonellae.

Slaughter samples were collected from two slaughterhouses. The first slaughter plant processed both the conventional and ABF pigs and used a blast chiller (−30°C for 2 h) for rapidly cooling the carcasses. The ABF pigs in this plant were processed only on the first day of every week and at the start of the first shift to prevent cross-contamination from conventionally reared pigs. Sixteen groups of pigs (11 conventional and five ABF) were processed in the first plant. This plant was also cleaned and disinfected every weekend to prevent contamination of carcasses. The second slaughter plant processed only ABF-reared pigs and used overnight chilling (1 to 4°C for approximately 18 h) for chilling of the carcasses. The remaining five ABF groups were processed at the second plant.

***Salmonella* isolation and identification.** *Salmonella* was isolated from fecal samples following the method described previously *(1).* For isolation from fecal sample, 10 g of the feces was dissolved in 90 ml of BPW (1:9, wt/vol) (Becton Dickinson) and incubated at 37°C for 24 h. Next, 100 μl of the suspension was selectively enriched in 9.9 ml of Rappaport-Vassilliadis media (Difco, Becton Dickinson, Sparks, Md.) and incubated at 42°C for 24 h. A loopful of the incubated media was transferred to XLT4 selective agar media (Difco, Becton Dickinson) and incubated at 37°C for 24 h. Up to five black-colored colonies from each positive sample were tested for the appropriate biochemical reactions on triple sugar iron and urea agar slants (Difco, Becton Dickinson). Confirmed *Salmonella* isolates were stored on Luria-Bertani agar slant (Difco, Becton Dickinson) until further characterization. For isolation of salmonellae from carcass swabs, individual bags with carcass swabs dipped in 10 ml of BPW were supplemented with an additional 20 ml of BPW upon arrival at the laboratory and incubated for 24 h at 37°C. One hundred microliters of the suspension was transferred to 9.9 ml of Rappaport-Vassilliadis media (Difco, Becton Dickinson) and incubated at 42°C for 24 h. The remaining isolation procedures were the same as the method used for isolation from fecal samples.

Antimicrobial susceptibility testing. The isolates were tested for susceptibility to 12 antimicrobial agents by the Kirby-Bauer disk-diffusion method. The antimicrobials tested and disk potencies used were ampicillin (AMP; 10 mg), amoxicillin–clavulanic acid (AMX; 30 mg), amikacin (30 mg), ceftriaxone (CRO; 30 mg), cephalothin (CEF; 30 mg), chloramphenicol (CHL; 30 mg), ciprofloxacin (5 mg), gentamicin (10 mg), kanamycin (KAN; 30 mg), streptomycin (STR; 10 mg), sulfamethoxazole (SUL; 250 mg), and tetracycline (TET; 30 mg). Results were interpreted according to 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 *(17, 18).*

Statistical analysis. To compare the prevalence, antimicrobial resistance profile, and patterns of *Salmonella* isolates between the two production systems, we used the chi-square test (Minitab Inc., Pa.) and Fisher's exact two-tailed test (www.matforsk.no/ola/fisher.htm) when applicable. An α of 0.05 was used as the significance level. To determine the strength of association between the different production systems and *Salmonella* occurrence as well as antimicrobial resistance to various classes of antimicrobial agents, the odds ratio (OR) with a 95% confidence interval (CI) was calculated. ?3

RESULTS AND DISCUSSION

***Salmonella* prevalence.** Initially, *Salmonella* prevalence on-farm and on carcasses at slaughter was determined. A total of 889 pigs and 743 carcasses were sampled during the study. Between the two production systems, *Salmonella* prevalence was significantly higher in the ABF (15.2%) than in the conventional system (4.2%) (OR = 4.23; $P < 0.05$). Similar to the on-farm findings, more *Salmonella* was recovered from the carcasses at slaughter from the ABF groups (15%) than from the conventional ones (6.8%), with an OR of 2.34. Overall *Salmonella* fecal prevalence was 10.7% ($n = 83$), and carcass swab prevalence was 9.3% ($n = 80$) ($P = 0.33$). Though not generalizable due to the limitation in geographic coverage and sampling design, the consistent findings of higher prevalence of *Salmonella* from ABF fecal samples on-farm could be a result of the absence of antimicrobial use in the ABF pigs. This finding is consistent with previous reports that show the increase in clinical infectious disease cases in countries where feed-grade antimicrobial use has been banned *(4).* Similar studies conducted in broilers in *Campylobacter* also show higher prevalence in ABF production units *(13).* In the current study, no serotyping data are available, and thus, we were not able to identify any association between specific serotypes and production systems, which is beyond the scope of the study. The higher occurrence of *Salmonella* in ABF production system, regardless of the serotype status, is interesting.

Antimicrobial resistance profiles among isolates from ABF and conventional herd. Antimicrobial resistance was detected against ten antimicrobials. The highest frequency of resistance was found against tetracycline (80%), followed by streptomycin (43.4%) and sulfamethoxazole (36%), irrespective of the production system or the production stage. This finding is consistent with previous findings in the study area *(11)* as well as in other geographic locations *(21).* Comparison of antimicrobial resistance between the conventional and ABF production systems for antimicrobials to which resistance was predominantly found is depicted in Table 1. Frequency of resistance to most classes of antimicrobials was significantly higher among con-

TABLE 1. *Comparison of antimicrobial resistance between* Salmonella *isolates from the conventional and antimicrobial-free (ABF) swine production systems*

Antimicrobial	Production system	On-farm isolation[a]			At-slaughter isolation[a]		
		No. resistant (%)	OR (95% CI)[b]	*P* value	No. resistant (%)	OR (95% CI)[b]	*P* value
Ampicillin	Conventional	30 (35.3)	3.95	<0.001	27 (23.5)	9.66	<0.0001
	ABF	35 (12.4)	(1.9–8.2)		7 (3.2)	(2.79–33.37)	
Amoxicillin/clavulanic acid	Conventional	12 (14.1)	16.1	<0.001	4 (3.5)	2.04	0.6
	ABF	2 (0.7)	(2.1–125)		4 (1.8)	(0.3–11.4)	
Ceftriaxone	Conventional	0 (0)	NA	NA	2 (1.7)	NA	NA
	ABF	0 (0)			0 (0)		
Cephalothin	Conventional	5 (5.9)	6.3	0.11	3 (2.6)	1.5	0.9
	ABF	1 (0.4)	(0.7–53.5)		5 (2.3)	(0.5–9.27)	
Chloramphenicol	Conventional	22 (25.9)	2.84	<0.01	25 (21.7)	56.1	<0.0001
	ABF	30 (10.6)	(1.32–6.14)		0 (0)	(3.4–940.5)	
Gentamicin	Conventional	2 (2.4)	NA	NA	0 (0)	NA	NA
	ABF	0 (0)			0 (0)		
Kanamycin	Conventional	16 (18.8)	23.22	<0.001	20 (17.4)	40.8	<0.001
	ABF	4 (1.4)	(3.0–177.2)		0 (0)	(2.41–688.9)	
Streptomycin	Conventional	73 (85.9)	10.92	<0.0001	63 (54.8)	2.85	<0.001
	ABF	102 (36.2)	(5.4–21.9)		67 (30.3)	(1.59–5.1)	
Tetracycline	Conventional	79 (93)	1.82	0.22	112 (97.4)	25.4	<0.0001
	ABF	249 (88.3)	(0.68–4.81)		123 (55.7)	(7.54–85.6)	
Sulfamethoxazole	Conventional	55 (64.7)	4.3	<0.0001	58 (50.4)	3	<0.001
	ABF	85 (30.1)	(2.39–7.84)		56 (25.3)	(1.65–5.46)	

[a] Eighty-five isolates from conventional and 282 isolates from ABF production systems were included in the on-farm analysis; 115 isolates from conventional and 221 isolates from AFB production systems were included in the at-slaughter analysis.

[b] OR, odds ratio indicates association of antimicrobial resistance with production system. CI, confidence interval. NA, not applicable.

ventional farms than ABF with ORs ranging from 2.84 for chloramphenicol to 23.22 for kanamycin at the on-farm level. There was no significant difference in the resistance against tetracycline between the two production systems at the farm level with an OR of 1.82, 95% CI (0.68 to 4.81), and $P = 0.22$. A very strong association was found between the conventional system and resistance to aminoglycosides streptomycin (OR = 0.92) and kanamycin (OR = 23.22). This was consistent with the reportedly common use of aminoglycoside in the conventional production, especially at the nursery. The wide occurrence of tetracycline resistance in both types of pig production and the discrepancy in findings between tetracycline and the other antimicrobials has two important implications. First, they illustrate that resistance to tetracycline is widespread and ubiquitous in various serovars spatially. Second, these findings, together with the discrepancy in findings on the other antimicrobials, indicate that the antimicrobial use and association with resistance is not uniform across various classes of antimicrobials, and thus, epidemiology and intervention policies need to be considered on a case-by-case basis.

The findings at slaughter had more variability than those on-farm as attested by the wide CIs of the OR to most antimicrobials except streptomycin and sulfamethoxazole (Table 1). In contrast to the findings on-farm, a very strong association between tetracycline resistance and the conventional production system was found at the slaughter level with an OR of 25.4 ($P < 0.001$). For the remaining classes of antimicrobials, the findings were consistent with those on-farm in that there was a significant association between resistance to various classes of antimicrobials and the conventional production system. The high variability among specimens from slaughter plants may be explained by the potential cross contamination, the diverse potential sources of *Salmonella* to pigs in the periharvest period, or a random error due to the limited sample size of this study. Resistance to amoxicillin–clavulanic acid and cephalothin was detected at a low frequency in both production types as shown in Table 1. *Salmonella* strains resistant to the third generation cephalothin ceftriaxone ($n = 2$) and gentamicin ($n = 2$) were also isolated from the conventionally reared pigs. None of the isolates from ABF slaughter pigs were resistant to these antimicrobials as well as chloramphenicol and kanamycin.

Phenotypes based on antimicrobial resistance patterns (R-types). A total of 28 antimicrobial resistance patterns were detected among the 703 *Salmonella* isolates on farm ($n = 367$; 85 from conventional and 282 from ABF) and at slaughter ($n = 336$; 115 from conventional and 221 from ABF). Among these, 10 patterns (depicted in Table 2) were commonly observed while the remaining 18 were observed in rare instances (with less than five isolates per production system and phase combinations). Resistance to tetracycline alone ($n = 273$) was the most common resistance pattern, followed by the multidrug resistance pattern, STR SUL TET ($n = 130$). As described previously, this pattern is often associated with the carriage of class 1 integrons in the genome of *Salmonella* and other *Enterobac-*

TABLE 2. *Predominant antimicrobial resistance patterns and association with respective swine production systems (antimicrobial-free [ABF] and conventional)*

Antimicrobial resistance pattern (n)[a]	On-farm isolation[b]				At-slaughter isolation[b]			
	No. in conventional system	No. in AFB system	OR[c] (95% CI)	P value	No. in conventional system	No. in AFB system	OR (95% CI)	P value
Pansusceptible (118)	1 (1.8)	33 (11.7)	0.15 (0.03–0.7)	0.005	0 (0)	84 (38)	0.01 (0.0–0.12)	<0.0001
TET (273)	9 (10.6)	146 (51.8)	0.11 (0.05–0.2)	<0.0001	50 (43.5)	67 (30.3)	1.75 (0.98–3.1)	>0.05
STR TET (36)	12 (14.1)	17 (6)	2.55 (0.93–6.9)	>0.05	0 (0)	7 (3.2)	0.33 (0.03–3.2)	>0.05
STR SUL (8)	5 (5.9)	0 (0)	5.2 (0.6–45.4)	>0.05	3 (2.6)	13 (5.8)	0.48 (0.12–1.99)	>0.05
STR SUL TET (130)	24 (28.3)	50 (17.7)	1.8 (0.9–3.47)	>0.05	15 (13)	41 (18.6)	0.64 (0.3–1.37)	>0.05
AMP CHL STR TET (5)	0 (0)	0 (0)	0 (0)	>0.05	5 (4.3)	0 (0)	4.1 (0.45–37.6)	>0.05
STR SUL TET KAN (20)	0 (0)	0 (0)	0 (0)	>0.05	20 (17.4)	0 (0)	20.3 (2.6–155.6)	<0.0001
STR TET KAN GEN (2)	2 (2.4)	0 (0)	2.0 (0.18–22.6)	>0.05	0 (0)	0 (0)	0 (0)	>0.05
AMP CHL STR SUL TET (48)	2 (2.4)	28 (9.9)	0.19 (0.04–0.92)	0.01	18 (15.6)	0 (0)	14.8 (1.9–15.4)	<0.001
AMP CHL STR SUL TET AMX (21)	18 (21.2)	2 (0.7)	26.3 (3.46–99.9)	<0.0001	1 (0.9)	0 (0)	2.0 (0.07–60.6)	>0.05
Other MDR patterns: 18 types (32)	12 (14.1)	6 (2.1)	7.9 (1.76–36.1)	0.001	3 (2.6)	9 (4)	0.74 (0.16–3.4)	>0.05

[a] AMP, ampicillin; AMX, amoxicillin/clavulanic acid; CHL, chloramphenicol; GEN, gentamicin; KAN, kanamycin; STR, streptomycin; SUL, sulfamethoxazole; TET, tetracycline.
[b] Eighty-five isolates from conventional and 282 isolates from AFB production systems were included in the on-farm analysis; 115 isolates from conventional and 221 isolates from AFB production systems were included in the at-slaughter analysis.
[c] OR, indicates the association between the resistance patterns with that of production system. CI, confidence interval.

tereaceae organisms *(11, 15).* There was no significant difference in the proportion of isolates with the STR SUL tetracycline pattern between the conventional (19.5%) and the ABF systems (18%) (OR = 1.8 on-farm and OR = 0.64 at slaughter; $P > 0.05$). This finding shows the occurrence of specific multidrug-resistant *Salmonella* strains regardless of the antimicrobial use levels of various pig production practices. A recent study on *Campylobacter* from poultry also concluded that fluoroquinolone resistance may persist in the commercial poultry environment in the absence of fluoroquinolone selective pressure *(19).*

Parallel to the findings on the antimicrobial resistance profiles, there was a significant and strong association between pansusceptibility and isolates from ABF samples (OR = 6.68 when ABF was used as the outcome variable in computing the OR; $P = 0.005$). At the farm level, a single isolate (1.8%) from the conventional production system was pansusceptible compared to 33 (11.7%) of the isolates from the ABF farms. The high level of pansusceptibility associated with ABF may in part be the result of absence of antimicrobial selective pressure in the ABF. On the other hand, some specific multidrug-resistant strains were able to persist in the ABF production units. On-farm isolates with a tetracycline resistance pattern were also highly associated with ABF groups (OR = 8.76 when ABF was used as the outcome variable in computing the OR; $P < 0.0001$). The AMP CHL STR SUL TET MDR pentaresistance pattern was seen in 48 (6.8%) of the isolates. One of the most striking findings in this study was that between the two production systems, this pentaresistance pattern was strongly associated with ABF groups with an OR of 5.4 (when ABF was used as the outcome variable in computing the OR) and $P = 0.01$ on-farm. This pentaresistance pattern has previously been shown to be associated with *Salmonella* serovar Typhimurium (*11*). In this study, identification of this pentaresistant pattern among ABF units was unexpected and interesting regardless of what serotype the isolates may belong to. Because this study was conducted on a limited number of farms and limited geographical areas, we cannot deduce a generalizable conclusion as to the dissemination of such a highly multidrug-resistant strain. However, these findings have important implications about the emergence and occurrence of multidrug-resistant strains that such strains could be introduced and persist despite the fact that no antimicrobials were used on the farms. Some potential explanations could be the introduction of MDR strains in the production units via other risk factors and coselection of MDR strains due to selective pressure other than direct antimicrobial use. Such phenomena have been suggested to occur with *Salmonella,* and the relationship between antimicrobial and nonantimicrobial compounds has been documented in other bacterial species *(12).* Other resistance patterns specific to a production system or production and/or processing phase were also noted, suggesting phenotypic diversity between different production systems as well as production and processing environments. Isolates with the MDR patterns STR SUL TET KAN (17.4%) and AMP CHL STR TET (4.3 %) were specific to a slaughterhouse that slaughtered the conventionally reared pigs and thus was isolated only from the carcasses of conventionally reared pigs. Other resistance patterns seen in isolates from the slaughter plants only (not shown on Table 2) included AMP STR AMX CEF (0.5%), AMP STR TET AMX CEF (0.5%), AMP TET AMX CEF CRO (1.7%), AMP CHL STR SUL TET AMX CEF (0.9%), and STR SUL TET CEF (0.5%).

The overall findings of this study show that antimicrobial resistance is to a large extent more common in conventional production units where antimicrobials were routinely used. However, distinct multidrug-resistant strains of *Salmonella* were also commonly detected in ABF herds. Such findings underscore the role of various risk factors that may enable persistence of resistant strains even in the absence of antimicrobial selective pressure.

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