Available online at www.sciencedirect.com
ScienceDirect
Veterinary Microbiology 125 (2007) 362–367
veterinary microbiology
www.elsevier.com/locate/vetmic

Short communication

Occurrence of multidrug resistant *Salmonella* in antimicrobial-free (ABF) swine production systems

Siddhartha Thakur [a], Daniel A. Tadesse [b], Morgan Morrow [c], Wondwossen A. Gebreyes [b,*]

[a] *Division of Animal and Food Microbiology, Office of Research, Center for Veterinary Medicine, U.S. Food and Drug Administration, Laurel, MD 20708, United States*
[b] *Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, 1920 Coffey Road, Columbus, OH 43210, United States*
[c] *College of Agriculture and Life Sciences, Department of Animal Sciences, North Carolina State University, 4700 Hillsborough ST., Raleigh, NC 27606, United States*

Received 15 February 2007; received in revised form 29 May 2007; accepted 30 May 2007

Abstract

This cross-sectional study was conducted to determine the prevalence and antimicrobial resistance of *Salmonella* species in swine reared in the intensive (indoor) and extensive (outdoor) ABF production systems at farm and slaughter in North Carolina, U.S.A. We sampled a total of 279 pigs at farm (extensive 107; intensive 172) and collected 274 carcass swabs (extensive 124; intensive 150) at slaughter. *Salmonella* species were tested for their susceptibility against 12 antimicrobial agents using the Kirby–Bauer disk diffusion method. Serogrouping was done using polyvalent and group specific antisera. A total of 400 salmonellae were isolated in this study with a significantly higher *Salmonella* prevalence from the intensive (30%) than the extensive farms (0.9%) ($P < 0.001$). At slaughter, significantly higher *Salmonella* was isolated at the pre- and post-evisceration stages from extensively (29% pre-evisceration and 33.3% post-evisceration) than the intensively (2% pre-evisceration and 6% post-evisceration) reared swine ($P < 0.001$). The isolates were clustered in six serogroups including B, C, E1, E4, G and R. Highest frequency of antimicrobial resistance was observed against tetracycline (78.5%) and streptomycin (31.5%). A total of 13 antimicrobial resistance patterns were observed including the pentaresistant strains with ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, tetracycline resistance pattern observed only among isolates from the intensive farms ($n = 28$) and all were serotype *Salmonella* typhimurium var. Copenhagen. In conclusion, this study shows that multidrug resistant *Salmonella* are prevalent in ABF production systems despite the absence of antimicrobial selection pressure. In addition, it also highlights the possible role played by slaughterhouse and other environmental factors in the contamination and dissemination of antimicrobial resistant *Salmonella* in ABF production systems.

Keywords: Swine; *Salmonella* species; Antimicrobial-free production system; Antimicrobials; Multidrug resistance

* Corresponding author. Tel.: +1 614 292 9559; fax: +1 614 292 4142.
E-mail address: gebreyes.1@osu.edu (W.A. Gebreyes).

0378-1135/$ – see front matter
doi:10.1016/j.vetmic.2007.05.025

1. Introduction

The FoodNet USA data for the year 2005 shows *Salmonella* with the highest overall incidence among bacterial foodborne pathogens with 14.55 cases per 100,000 USA population (Centers for Disease Control and Prevention, 2006). Swine have been shown to be colonized with different serovars of *Salmonella* and responsible for outbreaks in humans (Valdezate et al., 2005; Bucholz et al., 2005). Resistance to important antimicrobials has been reported previously in *Salmonella* isolated from swine reared in conventional production systems where antimicrobials are routinely used for growth promotion and treatment (Gebreyes et al., 2004). However, there is scarcity of information on the status of *Salmonella* in pigs that are reared in ABF systems including the outdoor (extensive) and indoor (intensive) systems. The primary objectives of this study were to determine the prevalence and the antimicrobial susceptibility of *Salmonella* isolates from the two types of ABF production systems at farm and slaughter.

2. Materials and methods

2.1. ABF production systems and sample collection

In all the ABF swine production systems included in the current study, no antimicrobials were used post-weaning. Even though the piglets were not directly exposed to antimicrobials, the sows were given parenteral antimicrobials. Therefore, it is possible that the piglets were exposed to antimicrobials indirectly through the sows. To keep the above observation in perspective, we adopted a conservative approach for defining the ABF production system as one where the pigs were not exposed to antimicrobials post-weaning. Under the extensive ABF system, pigs have free access to the environment and are placed in barricaded fields till slaughter. Pigs in the intensive system are placed in confined barns with concrete slatted floors. Approximately 10–12 pigs were grouped together in a single pen (space of 2.4 ft^2/pig at nursery and 7.4 ft^2/pig at finishing) under the intensively reared ABF production system. Under the extensive system, the pigs were reared in a barricaded area that was uncovered. These farms contained 40–50 pigs on an average.

Fecal samples were collected from five intensive and extensive finishing farms each over a period of 2 years from 2002 to 2004. Individual fecal samples (approximately 10 g) were collected per pig with gloved hands directly from the rectum and analyzed. Fecal samples were collected within 48 h of slaughter.

Pigs belonging to the two ABF systems were slaughtered at two different slaughter plants. Sterile swabs soaked in 10 mL of buffered peptone water (Becton Dickinson, NJ, USA) were swiped along the midline of the carcass extending from the ham to the jowl. Each group of 30 pigs sampled per farm was segregated in three groups of 10 pigs each. The first set of 10 pigs was sampled at pre-evisceration (immediately before evisceration of the gut), the next 10 pigs were sampled at post-evisceration (after gut evisceration) and the final set of 10 pigs was sampled at the post-chill stage (after the sample is chilled and ready for packing). The extensively reared pigs were slaughtered in a smaller slaughter plant (800 pigs processed/day) with the carcasses cooled overnight at 1–4 °C for 18 h. Pigs reared under the intensive system were processed in a large-scale plant (9000 pigs/day) and employed the modern blast chilling method (−30 °C) to cool the carcass surface within 2 h. Both the plants processed pigs from the conventional production systems as well. However, to avoid cross-contamination, the plants were cleaned with disinfectant over the weekend and the ABF pigs were processed separate from pigs from conventional system.

2.2. Salmonella isolation, serogrouping and serotyping

Salmonella isolation from the fecal samples and carcass swabs was done following the method described previously (Gebreyes et al., 2004, 2006). Briefly, 90 mL of buffered peptone water was mixed with 10 g of fecal material collected at the farm and incubated at 37 °C for 24 h. Next, 100 μL of the suspension was selectively enriched in 9.9 mL of Rappaport-Vassiliadis media (Difco) and incubated at 42 °C for 24 h. Loopful of the incubated media was transferred to XLT4 selective agar media (Difco) and incubated at 37 °C for 24 h. Multiple colonies (up to five) from each positive sample were tested on triple sugar iron (TSI) and urea agar media (Difco, Becton Dickinson) for biochemical testing. Confirmed *Salmonella* isolates were stored on

Luria-Bertani agar tubes (Difco, Becton Dickinson) till further characterization. Carcass swabs were first enriched in 20 mL of buffered peptone water (Becton Dickenson) and incubated at 37 °C for 24 h. The remaining steps for *Salmonella* isolation were the same as from fecal material. For serogrouping, *Salmonella* isolates were cultured overnight at 37 °C on Luria-Bertani (LB) agar and serogrouped using polyvalent and group specific antisera (Statens Serum Institut, Copenhagen, Denmark) following the recommendation of the manufacturer. *Salmonella* isolates were shipped to the National Veterinary Services Laboratories (NVSL) for serotyping.

2.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing for 12 antimicrobials was done using the Kirby–Bauer disk diffusion method. The antimicrobials tested and the disk potency used were: ampicillin (10 mg/L), amoxicillin–clavulanic acid (30 mg/L), amikacin (30 mg/L), ceftriaxone (30 mg/L), cephalothin (30 mg/L), chloramphenicol (30 mg/L), ciprofloxacin (5 mg/L), gentamicin (10 mg/L), kanamycin (30 mg/L), streptomycin (10 mg/L), sulfamethoxazole (250 mg/L) and tetracycline (30 mg/L). The MIC was determined and interpreted using the Clinical and Laboratory Standards Institute Standards (CLSI; formerly NCCLS) using appropriate quality control organisms (CLSI, 2006)

2.4. Statistical analysis

We used the χ^2 test (Minitab Inc. PA, USA) to compare the *Salmonella* prevalence, antimicrobial resistance profile and pattern between the two ABF systems. Strength of association between serogroup and resistance pattern as well as type of ABF system was determined using the odds ratio (OR) with a 95% confidence interval. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Salmonella prevalence

The overall *Salmonella* prevalence at the farm and slaughter was 24 and 15%, respectively with significantly higher prevalence at farm ($P < 0.001$). A single extensive ABF farm was positive for *Salmonella* ($n = 1$; 0.9%) compared to all the five intensive farms that tested positive ($n = 51$; 30%). At slaughter, contrary to the on farm findings, significantly higher prevalence was found from the extensive production system compared to the intensive system at both the pre-evisceration (29%) and post-evisceration (33.3%) stages ($P < 0.001$). There was no significant difference in prevalence between the two systems at the post-chill level ($P = 0.19$).

3.2. Antimicrobial resistance profile and patterns

A total of 71 isolates (17.7%) were pansusceptible. Resistance was observed against 8 of the 12 antimicrobials tested. Overall, the highest frequency of resistance was observed against tetracycline (78.5%) followed by streptomycin (31.5%) (Table 1). On comparing the two ABF systems at slaughter, significantly more isolates were resistant to sulfamethoxazole and tetracycline at all the three stages (pre-evisceration, post-evisceration, post-chill) among the extensively reared pigs ($P < 0.001$). Thirteen different resistance patterns were observed including 10 patterns that were multidrug resistant (MDR; resistant to $\geq$3 antimicrobials) (Table 2). Streptomycin, sulfamethoxazole, tetracycline were the most common MDR pattern (10.5%) and significantly more frequent in isolates from the carcass of extensively reared swine at all the three stages of slaughter ($P < 0.001$). Isolates with the pentaresistant MDR pattern ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, tetracycline were found from the intensive production system ($n = 28$). Frequency of MDR *Salmonella* isolation at slaughter was significantly higher among the extensively reared pigs ($P < 0.001$).

3.3. Serogrouping and serotyping

Among the 400 isolates, a total of six serogroups (B, C, E1, E4, G and R) and 13 untypable were found. Serogroup B was the most predominant observed in 174 (43.5%) isolates, including 50% of the isolates from the intensive production farm and slaughter levels ($n = 113$). Serogroup B was also the predominant serogroup observed in isolates ($n = 61$; 43%) from the extensive system. All the 28 isolates with the

Table 1
Antimicrobial resistance frequency comparison among the *Salmonella* isolates from extensive and intensive reared ABF pigs at farm and slaughter

Production stage	ABF system	Isolates tested	Number of isolates resistant to antimicrobials (%)[a]							
			AMP	CHL	STR	SXT	TET	AMX	CPH	KAN
Finishing farm	Extensive	1	1 (100)	0	1 (100)	0	1 (100)	0	1 (100)	0
slaughter	Intensive	226	31 (13.8)	30 (13.2)	64 (28.3)	48 (21.2)	202 (89.3)	2 (0.8)	0	1 (0.4)
Pre-evisceration	Extensive	43	0	0	24 (55.8)[1]	23 (53.4)[2]	17 (39.5)[3]	0	0	0
	Intensive	5	0	0	0	0	5 (100)	0	0	0
Post-evisceration	Extensive	73	3 (4)	0	24 (32)[1]	22 (29.3)[2]	57 (78)[3]	2 (2.6)	2 (2.6)	0
	Intensive	12	0	0	1 (10)	1 (10)	8 (66)	0	0	0
Post-chill	Extensive	25	4 (16)	0	9 (36)	5 (20)	19 (76)[4]	2 (8)	3 (12)	0
	Intensive	15	0	0	3 (20)	0	5 (33.3)[4]	0	0	0
Total isolates		400	39 (9.7)	30 (7.5)	126 (31.5)	99 (24.7)	314 (78.5)	6 (1.5)	6 (1.5)	1 (0.2)

For each antimicrobial, figures sharing common numerical superscripts were significantly different at $P < 0.05$ (Chi-square test and Fisher's exact two-tailed). No resistance was observed against AMK, amikacin; CRO, ceftriaxone; CIP, ciprofloxacin and GEN, gentamicin at any stage.

[a] Antimicrobials with number of isolates showing resistance against; percentage resistance is shown in parenthesis. AMP, ampicillin; AMX, amoxicillin/clavulanic acid; CPH, cephalothin; CHL, chloramphenicol; KAN, kanamycin; STR, streptomycin; SXT, sulfamethoxazole; TET, tetracycline.

pentaresistant MDR pattern ampicillin/chloramphenicol/streptomycin/sulfamethoxazole/tetracycline were clustered under serogroup B. Serotyping these 28 isolates showed they were all *S.* typhimurium var. Copenhagen. We did not find any association between serogroup B and production system (OR of 1.03; 95% CI 0.68–1.56). However, serogroup B was strongly associated with tetracycline resistant isolates ($n = 58$) from the intensive farms with an OR of 21.38, 95% CI (12.10–37.77).

4. Discussion

This study was conducted to determine the dynamics of *Salmonella* in swine population reared in ABF production system. Only a single pig from the extensive (outdoor) ABF system was positive for *Salmonella* compared to 51 from the intensive farms. Contrary to this finding, the risk of *Salmonella* infection in organic pigs reared outside has been shown to increase if the environment is contaminated (Jensen et al., 2006). Based on our finding, though prevalence on-farm was higher in intensive units, the risk of foodborne infection to humans was higher on products from extensive units as recovery of *Salmonella* from these herds was higher. This finding underscores the significance of pre-harvest and post-harvest cross-contamination. The low level of *Salmonella* isolation from extensive swine farms may be attributed to the fact that these farms were relatively newly established and the environment including soil and water were not exposed to high level of *Salmonella* shedding. Another possible explanation for this finding could also be a random error due to the limited sample size and origin of herds. Although the criterion for collecting samples from 30 pigs per herd was based on the power of the study ($P = 80\%$), the shortage of extensive ABF farms that we were able to recruit for sampling could be the reason for low *Salmonella* prevalence reported from these farms. On the other hand, higher prevalence of *Salmonella* and higher frequency of multidrug resistance was detected among intensively reared herd. This could be due to the fact that strains persist in the farm environment for very long period of time and also multiresistance may build up through time due to co-selection (Bolton et al., 1999).

The intensive farms were all-in all-out based system of production with the primary aim of reducing transmission of infectious agents such as *Salmonella* between different batches. However, *Salmonella* has been shown to persist on the farm floor of such systems even after it has been cleaned with disinfectants (Funk et al., 2001). A recent study conducted over a 2-year period to determine *Salmonella* prevalence in diverse environmental samples reported 57.3% of samples from swine production environment being positive for

Table 2
Predominant *Salmonella* antimicrobial resistance patterns comparison between the two ABF systems across farm and slaughter

Resistance pattern[a]	Production stage							
	Finishing farm[b]				Slaughter[c]			
	Extensive	Intensive	Pre-evisceration		Post-evisceration		Post-chill	
			Extensive	Intensive	Extensive	Intensive	Extensive	Intensive
Pansusceptible		24 (10.6)	13 (30.2)	–	15 (20)	4 (40)	5 (20)	10 (66.6)
TET	–	137 (60.6)	5 (11.6)	5 (100)	31 (42.4)	7 (58.3)	10 (40)	2 (13.3)
STR/TET	–	17 (7.5)	1 (2.3)	–	2 (4.6)	–	1 (4)	3 (20)
SXT/TET	–	–	14 (32.5)	–	1 (2.3)	–	–	–
AMP/STR/TET	–	–	–	–	1 (2.3)	–	2 (8)	–
AMP/AMX/CPH	–	–	–	–	1 (2.3)	–	1 (4)	–
STR/SXT/TET	–	6 (2.6)	10 (23.2)	–	21 (28)	1 (10)	4 (16)	–
SXT/TET/KAN	–	1 (0.4)	–	–	–	–	–	–
AMP/STR/SXT/TET	–	1 (0.4)	–	–	–	–	–	–
AMP/STR/AMX/CPH	1 (100)	–	–	–		–	1 (4)	–
AMP/TET/AMX/CPH	–	–	–	–	1 (2.3)	–	–	–
AMP/CHL/STR/SXT/TET	–	28 (12.3)	–	–	–	–	–	–
AMP/CHL/STR/SXT/TET/AMX	–	2 (0.8)	–	–	–	–	–	–
STR/SXT/TET/CPH	–	–	–	–	–	–	1 (4)	–
Total isolates	1	226	43	5	73	12	25	15

[a] Different resistance patterns shown with number of isolates; percentage resistance is shown in parenthesis. AMP, ampicillin; AMX, amoxicillin/clavulanic acid; CPH, cephalothin; CHL, chloramphenicol; KAN, kanamycin; STR, streptomycin; SXT, sulfamethoxazole; TET, tetracycline.

[b] Number of *Salmonella* isolates at farm: 1 (extensive) and 226 (intensive).

[c] Number of *Salmonella* isolates at slaughter: 141 (extensive) and 32 (intensive).

Salmonella (Rodriguez et al., 2006). Therefore, it is possible that the intensive ABF pigs get exposed to *Salmonella* once they are transferred to new farms as reflected in the significantly higher prevalence compared to the extensive farms. In addition, intensively reared pigs originated from a production pyramid system with those of conventional ones and are more closely confined which could help in the vertical and horizontal transmission of the pathogen.

High prevalence at extensive slaughter could be due to the slaughter plant effect. The slaughterhouses were not dedicated to ABF farms only and did process swine from conventional herds. Therefore, the potential cross-contamination existed at these slaughterhouses (Beloeil et al., 2004). We isolated *Salmonella* from the post-chill carcasses from both the ABF systems. This indicates that *Salmonella* is able to survive freezing temperatures, be it overnight or blast chilling.

Overall, the high frequency of antimicrobial resistance seen in *Salmonella* isolates without antimicrobial selection pressure indicates other sources of transmission. This was clearly illustrated in 13.2% chloramphenicol resistant isolates from the intensive farms. Chloramphenicol has not been used in any swine production system for the last two decades. This shows that antimicrobial resistant *Salmonella* can exist in the environment even in the absence of selection pressure and have the potential to transmit to other swine over a long period of time. We observed specific resistance patterns that were observed only at slaughter (Table 2). It is possible that these isolates were either not isolated at the farm level, were shed at slaughter under increased stress or were transmitted at large. Few MDR patterns were observed only in isolates from the slaughter plant suggesting phenotypic diversity based on the stage of sample processing.

The predominant pentaresistant pattern ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, tetracycline was seen only in isolates from the intensive farm. High frequency of resistance to these classes of antimicrobials in pigs reared under intensive farms has been reported previously (Nollet et al., 2006). Previous studies conducted in the same geographical region on conventional farms have shown this pattern to be associated with *S.* typhimurium DT 104 phage types (Gebreyes et al., 2004, 2006). This was indeed the case

with the 28 MDR strains isolated from the intensive farms (R-ACSSuT) as they were all *S.* typhimurium var. Copenhagen. Based on the serotype and antimicrobial susceptibility profile, it is possible that these isolates are *S.* typhimurium DT 104

This study shows that MDR *Salmonella* strains exist in the ABF production system both at farm and slaughter even in the absence of the antimicrobial selection pressure and has important implications from food safety perspective. We recommend conducting detailed epidemiological based studies to determine the role played by environment in dissemination of *Salmonella* in swine reared in ABF production systems.

Acknowledgement

This work was supported by a research grant by the National Pork Board to Wondwossen Gebreyes.

References

Beloeil, P.A., Chauvin, C., Proux, K., Madec, F., Fravalo, P., Alioum, A., 2004. Impact of *Salmonella* status of market-age pigs and the pre-slaughter process on *Salmonella* caecal contamination at slaughter. Vet. Res. 35, 513–530.

Bolton, L.F., Kelley, L.C., Lee, M.D., Fedorka-Cray, P.J., Maurer, J.J., 1999. Detection of multidrug-resistant *Salmonella* enterica serotype Typhimurium DT104 based on a gene which confers cross-resistance to florfenicol and chloramphenicol. J. Clin. Microbiol. 37, 1348–1351.

Bucholz, U., Brodhun, B., Brockmann, S.O., Dreweck, C.M., Prager, R., Tschape, H., Ammon, A., 2005. An outbreak of *Salmonella* Muenchen in Germany associated with raw pork meat. J. Food Prot. 68, 273–276.

Centers for Disease Control and Prevention (CDC), 2006. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food-10 States, United States, 2005. Morb. Mortal. Wkly. Rep. 55, 392–395.

Clinical and Laboratory Standards Institute, 2006. Performance Standards for Antimicrobial Disk Susceptibility Tests: Approved Standard, Ninth ed. CLSI, Wayne, PA, USA (M2-A9).

Funk, J.A., Davies, P.R., Gebreyes, W.A., 2001. Risk factors associated with *Salmonella* enterica prevalence in three-site swine production systems in North Carolina, USA. Berl. Munch. Tierarztl. Wochenschr. 114, 335–358.

Gebreyes, W.A., Thakur, S., Davies, P.R., Funk, J., Altier, C., 2004. Trends in antimicrobial resistance, phage types and integrons among Salmonella serotypes from pigs, 1997–2000. J. Antimicrob. Chemother. 53, 997–1003.

Gebreyes, W.A., Thakur, S., Morrow, W.E.M., 2006. Comparison of prevalence, antimicrobial resistance and occurrence of multidrug-resistant salmonella in antimicrobial-free and conventional pig production. J. Food Prot. 69, 743–748.

Jensen, A.N., Dalsgaard, A., Stockmarr, A., Nielsen, E.M., Baggesen, D.L., 2006. Survival and transmission of Salmonella enterica Serovar Typhimurium in an outdoor organic pig farming environment. Appl. Environ. Microbiol. 72, 1833–1842.

Nollet, N., Houf, K., Dewulf, J., Catry, B., De Zutter, L., De Kruif, A., Maes, D., 2006. Variability in antimicrobial resistance among Salmonella enterica strains from fattening pigs and sows. Microb. Drug Resist. 12, 74–81.

Rodriguez, A., Pangloli, P., Richards, H.A., Mount, J.R., Draughon, F.A., 2006. Prevalence of Salmonella in diverse environmental farm samples. J. Food Prot. 69, 2576–2580.

Valdezate, S., Vidal, A., Herrera-Leon, S., Pozo, J., Rubio, P., Usera, M.A., Carvajal, A., Echeita, M.A., 2005. Salmonella Derby clonal spread from pork. Emerg. Infect. Dis. 11, 694–698.