

Comparison of multidrug resistant *Salmonella* between intensively- and extensively-reared antimicrobial-free (ABF) swine herds

Thakur, S. ⁽¹⁾, Tadesse, D. ⁽²⁾, Morrow, M. ⁽³⁾, Gebreyes, W. ^{*(2)}

⁽¹⁾ Division of Animal and Food Microbiology, Office of Research, Center for Veterinary Medicine, U. S. Food and Drug Administration, 20708, Laurel, Maryland, USA

⁽²⁾ Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, 1920 Coffey Road, 43210, Columbus, Ohio, USA

⁽³⁾ College of Agriculture and Life Sciences, Department of Animal Sciences, North Carolina State University, 4700 Hillsborough Street, 27606, Raleigh, North Carolina, USA
corresponding author: gebreyes.1@osu.edu

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 belonged to serogroup B. This study shows that multidrug resistant *Salmonella* are prevalent in ABF production systems despite the absence of antimicrobial selection pressure.

Introduction

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.

Materials and methods

In all the ABF swine production systems included in the current study, no antimicrobials were used 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. We collected approximately 10 grams of fresh faecal samples per rectum with gloved hands from 30 pigs within 48 hours of slaughter. Ten individual carcass swabs were collected at each of three processing stages: pre-evisceration, post-evisceration and post-chill. 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 hours. Pigs reared under the intensive system were processed in a large scale plant (9,000 pigs/ day) and employed the modern blast chilling method (-30 °C) to cool the carcass surface within two hours. 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. *Salmonella* isolation from the fecal samples and carcass swabs was done following the method described previously (Gebreyes et al., 2004, 2006). 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. Serogrouping was done following the manufacturer recommendations (Statens Serum Institut, Copenhagen, Denmark). Antimicrobial susceptibility testing and MIC determination for 12 antimicrobials were done using the Kirby-Bauer disk diffusion method as described previously (Gebreyes et al., 2006; NCCLS, 2002). 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.

Results

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 pig from the extensive ABF farm was positive for *Salmonella* compared to 51 (30%) pigs from the intensive farms. At slaughter, in contrast to the on-farm findings, significantly higher prevalence was found from the extensive production 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 ($P = 0.19$).

Resistance was observed against eight of the 12 antimicrobials tested. Overall, the highest frequency of resistance was observed against tetracycline (78.5%) followed by streptomycin (31.5%) (Table 1).

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	Number of isolates resistant to antimicrobials (%) ^a								
		Isolates Tested	AMP	CHL	STR	SXT	TET	AMX	CEF	KAN
Finishing Farm	Extensive	1	1 (100)	0	1 (100)	0	1 (100)	0	1 (100)	0
	Intensive	226	31 (13.8)	30 (13.2)	64 (28.3)	48 (21.2)	202 (89.3)	2 (0.8)	0	1 (0.4)
Slaughter										
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)

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

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 three antimicrobials). 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 ($P < 0.001$). A total of 71 isolates (17.7%) were pansusceptible.

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 found in 174 (43.5%) isolates. All the 28 isolates with the pentaresistant MDR pattern ampicillin/chloramphenicol/streptomycin/sulfamethoxazole/tetracycline were clustered under serogroup B. 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).

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 periharvest and postharvest 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.

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 two year period to determine *Salmonella* prevalence in diverse environmental samples reported 57.3% of samples from swine production environment being positive for *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 slaughter houses 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 lairage. 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. This pattern is commonly observed among *S. Typhimurium* DT 104 strains which are commonly associated with the presence of Class I integrons. 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). It is not possible to conclude whether these isolates are DT 104 since we did not serotype and phage type them. However, *S. Typhimurium* DT 104 belongs to serogroup B and all the 28 isolates with this pattern in this study were clustered under serogroup B. It is therefore possible that these isolates are *S. Typhimurium* DT 104.

Conclusions

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.

References

- BELOEIL, P., et al., 2004. Impact of *Salmonella* status of market-age pigs and the pre-slaughter process on *Salmonella* caecal contamination at slaughter. *Veterinary Research*, 35 (5), 513-530.
- BUCHOLZ, U., et al., 2005. An outbreak of *Salmonella* Muenchen in Germany associated with raw pork meat. *Journal of Food Protection*, 68 (2), 273-276.
- FUNK, J., et al., 2001. Risk factors associated with *Salmonella enterica* prevalence in three-site swine production systems in North Carolina, USA. *Berliner und Münchener tierärztliche Wochenschrift*. 114 (9-10), 335-358.
- GEBREYES, W., et al., 2004. Trends in antimicrobial resistance, phage types and integrons among *Salmonella* serotypes from pigs, 1997-2000. *Journal of Antimicrobial Chemotherapy*, 53 (6), 997-1003.
- GEBREYES, W., et al., 2006. Comparison of Prevalence, Antimicrobial Resistance and Occurrence of Multidrug-Resistant *Salmonella* in Antimicrobial-Free and Conventional Pig Production. *Journal of Food Protection*, 69, 743-748.
- JENSEN, A., et al., 2006. Survival and transmission of *Salmonella enterica* Serovar Typhimurium in an outdoor organic pig farming environment. *Applied Environmental Microbiology* 72 (3), 1833-1842.
- NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS, 2002. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals- Second Edition: Approved Standard M31-A2. NCCLS, Wayne, PA, USA, 2002.
- RODRIGUEZ, A., et al., 2006. Prevalence of *Salmonella* in diverse environmental farm samples. *Journal of Food Protection*, 69 (11), 2576-2580.
- VALDEZATE, S., et al., 2005. *Salmonella* Derby clonal spread from pork. *Emerging Infectious Diseases*, 11 (5), 694-698.