ISSN: 1511-3701
Pertanika J. Trop. Agric. Sci. **34 (2)**: 303 - 310 (2011)

The Prevalence of Antimicrobial Resistant *Salmonella* spp. and the Risk Factors Associated with Their Occurrence in Finisher Pigs in Seberang Perai, Malaysia

D.W. Choe[1], L. Hassan[2] and T.C. Loh[1,3*]
[1]Department of Animal Science, Faculty of Agriculture,
[2]Department of Veterinary Pathology and Microbiology,
Faculty of Veterinary Medicine,
[3]Institute of Tropical Agriculture,
Universiti Putra Malaysia,
43400 UPM, Serdang, Selangor, Malaysia
[*]E-mail: tcloh@upm.agri.edu.my

ABSTRACT

A cross sectional study to determine the occurrence and antimicrobial resistance pattern of *Salmonella* spp. in finishing pigs was carried out at 12 selected pig farms. The farm characteristics and certain management practices associated with the occurrence of *Salmonella* spp. were also evaluated. Rectal swabs were collected aseptically from 210 randomly selected finishing pigs. Suspect *Salmonella* colonies isolated were identified using a set of conventional biochemical tests and these isolates were sent to the Veterinary Research Institute (VRI) for serotyping. The antimicrobial sensitivity test was conducted on the isolates against a panel of selected antimicrobials that are commonly used in local pig production using disc diffusion method. In addition, a questionnaire on the management of farms, herd health programme, and common antimicrobial usage was collected from farm representative, resulting in variables that could be analyzed to identify factors associated with the occurrence of *Salmonella.* The results showed that 32 out of 210 finishing pigs (15.2%) tested were positive for *Salmonella typhimurium* and the isolates were detected from 9 out of 12 farms sampled (75.0%). Most of the isolates showed a relatively high level of antimicrobial resistance. The occurrence of *Salmonella* in pigs were significantly associated with (1) farms which were not equipped with footbath or vehicle wheels dip; (2) farms with vermins/birds in the surrounding; (3) farms with less frequent pen cleaning and (4) farms which are located adjacent to the neighbouring farms.

Keywords: *Salmonella*, finishing pigs, prevalence, antimicrobial resistance pattern, risk factors

INTRODUCTION

Members of the genus *Salmonella* are known for their capability to infect a broad range of host, which is considered to be a major feature in their success as food pathogens. Taylor & McCoy (1969) reported that *Salmonella* have been isolated from virtually all vertebrates from which they have been sought, with the possible exception of fish in uncontaminated waters.

Currently, more attention has been directed to *Salmonella* infection, specifically in pork and pig productions for two reasons. Firstly, in the 1990's, there was an increase in the surveillance of food-borne pathogens, giving the impression that the levels of *Salmonella* in meat products

Received: 21 July 2010
Accepted: 10 November 2010
[*]Corresponding Author

had considerably increased. This coincided with more attention to the emergence of virulent strains and to antibiotic resistance *Salmonella*. Secondly, from the swine medicine point of view, there have been more reports of the outbreaks of clinical *Salmonella* infection in healthy finishing pigs reared even in well-run, sanitary barns (Paul, 2002). It is important to note that preventing and limiting *Salmonella* infection in the animals at farm-level are essential, as the detection of *Salmonella* infected animals is made difficult by the absence of clinical signs. Therefore, the production of *Salmonella*-free finisher pigs is necessary to ensure safe and consumable pork and pork products (Crump *et al*., 2002).

Antibiotics kill most, if not all, of the susceptible bacteria causing an infection but leave behind the bacteria that have developed resistance. In fact, antibiotics have widely been prescribed to treat bacterial infections in humans, while many antibiotics that are commonly used in humans have also been used in animals for therapeutic and other purposes, including growth enhancement. Resistance to penicillin, a broad spectrum, started to emerge soon after its extensive introduction (Matthew *et al*., 2007). Since then, resistance to other antibiotics has also emerged.

It is a necessity to conduct epidemiological studies to determine the prevalence and possible risk factors so that *Salmonella* can be monitored and controlled at all levels of pig production. Therefore, this study was carried out to determine the occurrence of *Salmonella* in the finishing pigs, and the level of antibiotic resistance of the isolated *Salmonella* spp., as well as to identify certain management practices associated with the occurrence of *Salmonella*.

MATERIALS AND METHODS

Study Design

A small scale cross sectional study was conducted to address the objectives of the study. A total of 12 pig farms from six different pig farming areas in Seberang Perai, Pulau Pinang, were randomly selected and visited with the help of an enforcement officer from MPSP (Majlis Perbandaran Seberang Perai). The number of farms and the total of animals chosen were based on practical and financial reasons. All the farms are located at the pig farming area in Seberang Perai and the farm owners had agreed to collaborate in the present study (100% response rate).

Samples Collection and Transportation

Rectal swabs from the finisher pigs were collected aseptically using gloved hands. A total of 210 finisher pigs were randomly sampled, and about 15 to 20 rectal swabs were obtained from the finisher pigs in each farm. Each sample was inoculated immediately into a bottle containing 2 ml buffered peptone water (BPW), and kept in an ice chest containing ice-packs to maintain the survivability of the bacteria in the swab (Nielsen *et al*., 1997).

Herd Data

During the farm visits, information such as: (1) total standing pig population (SPP); (2) total and age of finisher pigs; (3) source of pigs and the management practices like: (1) total workers; (2) frequency of cleaning; (3) herd health programme, and (4) common antimicrobial usage were collected using a questionnaire. A number of closed-questions with pre-fixed answers were developed and a face-to-face interview was conducted after the samples had been collected from each farm.

Bacteriological Analysis

Each sample was pre-enriched in 2ml of BPW (Oxoid Ltd., UK) and incubated at 37°C for 18 to 24 hours. From the pre-enrichment broth, 1 ml of the media was transferred into 10 ml of Rappaport-Vassiliadis (Oxoid Ltd., UK) medium (1:10 dilution) and further incubated for 18 to 24 hours at 37°C. All the samples were then plated onto xylose lysine desoxycholate (XLD, Merck) agar and xylose lysine tergitol 4 (XLT_4,

Merck). Presumptive *Salmonella* colonies were characterised using a set of biochemical tests (Table 1) and were further confirmed by slide agglutination test using Polyvalent 'O' and 'H' antisera (Difco). The confirmed colonies were sub-cultured onto a nutrient agar slant by streaking onto its surface. The cultures were sent to VRI for serotyping (Maria *et al*., 2002).

Antimicrobial Sensitivity Test

The antimicrobial susceptibility test of *Salmonella* isolates was conducted against a panel of 25 commonly selected antimicrobials using Kirby-Bauer disc diffusion method: amikacin (30µg), amoxicillin (25µg), amoxicillin/clavulanate (30µg), ampicillin (10µg), apramycin (15µg), carbenicillin (100µg), cephalothin (30µg), chloramphenicol (30µg), ciprofloxacin (5µg), clindamycin (2µg), cephradine (30µg), cephalexin (30µg), collistin sulphate (10µg), enrofloxacin (5µg), fosfomycin (50µg), gentamycin (10µg), kanamycin (30µg), neomycin (30µg), norfloxacin (10µg), pefloxacin (5µg), tetracycline (30µg), penicillin (10µg), sulphamethaxazole (25µg), oxytetracycline 30µg, and chlortetracycline 30µg. The susceptible and resistance breakpoint levels of the antimicrobials were based mainly on those specified by the Clinical and Laboratory Standards Institute (CLSI, 2006). The inhibition zones of each antibiotic were measured after 24 hours of incubation at 37°C.

Statistical Analysis

All data were stored in SPSS v15.0 (STATCON, Witzenhause, Germany). Meanwhile, data quality was evaluated and obvious typing errors were checked against the original records and corrected. The statistical analyses involved screening of all single explanatory categorical variables by χ^2 or Fisher Exact test. Strength of association between *Salmonella* occurrence and significant risk factors were determined using the odds ratio (OR) with a 95% confidence interval. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Isolation of Salmonella *spp.*

Among a total of 210 finisher pigs, 32 pigs were found to be positive for *Salmonella* spp. The overall prevalence of *Salmonella* spp. isolated from the finisher pigs was 15.2%. The organism was isolated from nine out of twelve (75%) farms. Serotyping of the isolates in Veterinary Research Institute revealed that all the isolates were identified as *Salmonella* typhimurium.

Antimicrobial Resistance Pattern

The resistance pattern of *Salmonella typhimurium* isolated from the finisher pigs is presented in Table 2. A total of 25 antimicrobials were used to test against the *Salmonella* spp. isolated from this study. All the isolates were resistant

TABLE 1
Biochemical tests for the identification of *Salmonella* spp.

Biochemical test	Results
Triple Sugar Iron test	O/AG H_2S gas+/-
Urease test	Negative
Phenylalanine Deaminase test	Negative
Citrate test	Positive/ Negative
Arginine	Positive/ Negative
Ornithine	Positive
Lysine	Positive
Polyvalent O	Positive
Polyvalent H	Positive/ Negative

TABLE 2
Percentage of antimicrobial sensitivity in the *Salmonella* spp. (n=32) isolated from 210 finisher pigs

Antimicrobials	Potency	Sensitive	Intermediate	Resistant
		(%)	(%)	(%)
Amikacin (AMK)	30µg	32(100.0)	-	-
Amoxycillin (AML25)	25µg	7(21.8)	18(56.4)	7(21.8)
Amoxy/Clavu (AMC30)	30µg	32(100.0)	-	-
Ampicillin (AMP10)	10µg	21(65.6)	4(12.5)	7(21.8)
Apramycin (APR)	15µg	32(100.0)	-	-
Carbenicillin (CAR100)	100µg	11(34.4)	17(53.1)	4(12.5)
Cephalothin (CF30)	30µg	7(21.8)	11(34.4)	14(43.8)
Cephalexin (CL30)	30µg	32(100.0)	-	-
Cephradine (CE30)	30µg	-	21(65.6)	11(34.4)
Chloramphenicol (C30)	30µg	-	4(12.5)	28(87.5)
ChlorTetracycline	30µg	-	4(12.5)	28(87.5)
Ciprofloxacin (CIP5)	5µg	25(78.2)	7(21.8)	-
Clindamycin (DA2)	2µg	-	-	32(100.0)
Colistin sulphate (CL)	10µg	28(87.5)	-	4(12.5)
Enrofloxacin (ENR)	5µg	21(65.6)	11(34.4)	-
Fosfomycin (FOS50)	50µg	32(100.0)	-	-
Gentamicin(CN10)	10µg	32(100.0)	-	-
Kanamycin (K)	30µg	32(100.0)	-	-
Neomycin (N30)	30µg	11(34.4)	21(65.6)	-
Norfloxacin (Nor10)	10µg	32(100.0)	-	-
OxyTetracycline	30µg	-	4(12.5)	28(87.5)
Penicillin (P10)	10µg	-	-	32(100.0)
Pefloxacin (PEF5)	5µg	-	18(56.2)	14(43.8)
Sulphamethoxazole (SOX25)	25µg	-	-	32(100.0)
Tetracycline (TE30)	30µg	-	-	32(100.0)

to at least four of the antimicrobial tested. The highest frequency of resistance was detected for towards penicillin (100%), tetracycline (100%), clindamycin (100%), and sulphamethoxazole/ trimethoprim (100%). 87.5% of the isolates tested were resistant towards chloramphenicol, oxytetracycline and chlortetracycline. Meanwhile, lower percentages of resistance were observed towards cephalothin (43.8%), pefloxacin (43.8%), amoxycillin (21.8%), ampicillin (21.8%), carbenicillin (12.5%), and collistin sulphate (12.5%). 100% of the isolates tested were shown to be completely sensitive towards amikacin, amoxycillin/ clavulanate, apramycin, fosfomycin, gentamicin, kanamycin, norfloxacin, and cephalexin (8 out of 25 antimicrobials tested). More than 90.0% of the isolates were resistant to more than five antimicrobials used in the antimicrobial sensitivity test (see Table 3). Various studies have reported similar findings, where high percentages of multiple antimicrobial resistant *Salmonella* spp. were isolated from rectal swabs in pigs (Kishima *et al*., 2008; Pan *et al.*, 2010) and in other domestic species such as chicken and ducks (Tran *et al*., 2004).

Risk Factors

The analysis of the herd data showed that there were a few possible risk factors in the farm management practices, and these

TABLE 3
Multidrug resistance observed among the *Salmonella* isolates obtained from 210 finisher pigs

Salmonella Isolates (no)	Percentage of resistant to indicated number of antimicrobials				
	1-5	6-10	11-15	>16	Total (>1)
S. Typhimurium (n = 32)	9.4	34.4	43.7	12.5	100.0

might be associated with the occurrence of *S. typhimurium* (n = 32) isolated from the sampled farms (Table 4). A significant higher number of *S. typhimurium* was isolated from the farms which were not equipped with vehicle dip (χ^2= 6.65, p<0.05, OR=2.7) or footbath (χ^2= 8.263, p<0.05, OR=3.0) compared to the farms having vehicle dip or footbath. A significant number of *Salmonella* were isolated from the pigs sampled from the farms in which vermin or birds were observed in the vicinity (χ^2=3.901, p<0.05, OR=2.2). More *Salmonella* was isolated from the farms where cleaning of pens was performed only once daily (χ^2=5.422, p<0.05, OR=2.8) compared to the ones where cleaning was done more than once a day. Higher occurrence of *Salmonella* was associated with farms situated adjacent to other farms (χ^2= 4.778, p<0.05, OR=3.2).

In addition, the statistical analysis showed that farm characteristics such as: (1) the age of farm; (2) total standing pig population, and (3) total finishing pigs, as well as management practices like: (1) total number of workers in the farm; (2) age when pig is marketed; (3) source of feed; (4) source of water, and (5) frequency of feeding in a day were not associated (p>0.05) with the occurrence of *Salmonella* spp.

DISCUSSION

Salmonella typhimurium was isolated from 15.2% of the finisher pigs sampled in this study. However, the finding is inconsistent with the

TABLE 4
Significant risk factors associated with the occurrence of *Salmonella* spp.

Significant risk factors	X^2	OR[a]	95% CI[b]	p value
Availability of footbaths				
Yes		1[c]		
No	8.2663	3.0	1.44-7.15	0.004
Availability of vehicle dips				
Yes		1[c]		
No	6.65	2.7	2.34-8.30	0.010
Presence of vermin/birds				
Yes	3.901	2.2	2.03-6.75	0.048
No		1[c]		
Cleaning of pens				
Once or less	5.422	2.8	1.15-6.78	0.020
More than once		1[c]		
Location of the farm				
Adjacent to other farms/residency	4.778	3.2	1.08-9.59	0.029
Distant from other farms/residency		1[c]		

[a] OR= Odds ratio
[b] 95%CI= 95% Confidence interval
[c] Reference category

result by a study by Wondwossen *et al.* (2006), where a lower percentage of faecal *Salmonella* (4.2%) was found in conventional pig farms. This could be due to the geographical variation and the differences in the production system. *Salmonella typhimurium* have been associated with multidrug resistance, which was a major reason in its success as pathogen (Schwartz *et al.*, 1999). Therefore, their potential for survivability is much higher compared to other serotypes, specifically in adult pigs.

A high percentage of the isolates tested in this study were found to be resistant towards penicillin, tetracyline, oxytetracycline, chlortetracycline, chloramphenicol, and sulphamethoxazole. The finding is in agreement with that by Wondwossen *et al.* (2006) who found high resistance in antimicrobials such as penicillin, tetracycline, and sulpha drugs. This was most probably due to improper usage of a particular antimicrobial causing resistance to occur. A lower resistance was exhibited by *Salmonella* isolates towards carbenicillin, colistin sulphate, ampicillin, amoxycillin, cephradine, and flumequine. This could be due to the lower frequency of usage of the newly established antimicrobials in the farms which might have further reduced the risk of antimicrobial resistance. All the isolates tested were shown to be sensitive towards 8 out of 25 (amikacin, amoxicillin/clavulanate, apramycin, cephalexin, fosfomycin, gentamycin, kanamycin, and norfloxacin) antimicrobials tested. This might be due to the less usage of these antimicrobials, which in turn, did not cause any emergence of resistant *Salmonella*.

In the present study, all *S. typhimurium* isolated were resistant to at least four antimicrobials, suggesting that the multidrug-resistant (MDR) strains of *Salmonella* are prevalent. MDR can be defined as a condition where bacteria or any disease causing organism gain resistance towards the clinical doses of classical antibiotics which were previously effective against them (Cowen, 2008). The MDR strains of *Salmonella* are now frequently encountered and the rates of multidrug-resistance have considerably increased in the recent years (Paul, 2002). Some variants of *Salmonella* (e.g. phage type of *S. typhimurium*) have developed multidrug-resistance as an essential part of the genetic material of the organism, and are therefore likely to preserve their drug-resistant genes and enhance their survivability (Rowe *et al*., 1997).

The current study found that higher *Salmonella* prevalence was associated with farms, which were not equipped with vehicle dip or footbaths. During transportation of pigs and feed (Berends *et al.*, 1996), *Salmonella*-negative finishing pigs might be infected from the previously contaminated trucks that had not been thoroughly cleaned (Fedorka-Cray *et al.*, 1997). Meanwhile, the presence of vermins and birds could be a source of *Salmonella* transmission, shedding and contaminating the farm and the feed with *Salmonella* (Davies & Wray, 1997; Funk *et al*., 2001). Therefore, controlling birds and vermins in the farm, as well as keeping pets, such as cats and dogs out of the vicinity, will help to prevent the introduction of *Salmonella* into the farm (Letellier *et al*., 1999).

The present study have also shown that a high number of *Salmonella* could be isolated from pigs where cleaning of the pens were done less frequently. Mannion *et al*. (2007) reported that high prevalence farms tended to have more residual contamination of feeders and equipment after barn cleaning than low prevalence farms, suggesting that more stringent and frequent cleaning could be associated with decreased prevalence. Therefore, pens should be cleaned more frequently to avoid accumulation of *Salmonella* in the environment. *Salmonella* could be transmitted through dust (Baggesan *et al.*, 1996; Edel *et al.*, 1970) and aerosols (Lever & Williams, 1996) which might have increased the risk of *Salmonella* transmission in farm located adjacent to other neighbouring farms. Furthermore, the movement of individuals or farm personnel between the farms might also increase the risk of *Salmonella* contamination.

CONCLUSIONS

The high prevalence of *Salmonella* in the local pig farms and the level of antimicrobial resistance in *Salmonella* have raised concerns and constituted a real threat to the public health. Thus, proper drug therapy practices and high hygiene standard in the farm can considerably reduce *Salmonella* contamination in the farm, and these will ultimately benefit the consumers. The findings in this study may not necessarily reflect the general contemporary production environments as the epidemiology of *Salmonella* infection in swine farm is very complex. However, these results may be used as a basis to show that pigs may pose as potential reservoir in harbouring multidrug-resistant *Salmonella*. More research is still needed to identify the possible risk factors associated with the occurrence of *Salmonella* spp. in pig farms so that intervention can be done to keep *Salmonella* levels low. This study reinforces the importance of good farm management and prudent use of antimicrobials to control the disease at all levels of animal production effectively.

REFERENCES

Baggesen, J. D., Wingstrand, A., & Nielsen, B. (1996). Critical control points in pig herds in relation to subclinical *Salmonella* infection (pp. 171).

Berends, B.R., Urlings, H.A.P., Snijders, J.M.A., & Van Knapen, F. (1996). Identification and quantification of risk factors in animal management and transport regarding *Salmonella* spp. in pigs. *International Journal of Food Microbiology*, *30*, 37-53.

CLSI. (2006). Performance standards for antimicrobial susceptibility testing, 15th Informational Supplement M100-S16. 16th Informational Supplement. Clinical and Laboratory Standards Institute, Wayne, Pa, USA.

Cowen, L.E. (2008). The evolution of drug resistance: Modulating the trajectory from genotype to phenotype. *Nature Reviews Microbiology*, *3*, 187-98.

Crump, J.A., Griffin, P.M., & Angulo, F.J. (2002). Bacterial contamination of animal feed and its relationship to human foodborne illness. *Clinical Infection Disease*, *35*, 859-865.

Davies, R.H., & Wray, C. (1997). Distribution of *Salmonella* contamination in ten animal feedmills. *Journal of Veterinary Microbiology*, *57*, 159-169.

Edel, W., van Schothorst, M., Guinee, P.A.M., & Kampelmacher, E.H. (1970). Effect of feeding pellets on the prevention and sanitation of *Salmonella* infections in fattening pigs. *Journal of Veterinary Medicine*, *17*, 730-738.

Fedorka-Cray, Hogg, A., Gray, J.T., Lorenzen, K., Velasquez, J., & Von Behren, P. (1997). Feed and feed trucks as sources of *Salmonella* contamination in swine. *Swine Health Production*, *5*, 189-193.

Funk, J.A., Davies, P.R., & Gebreyes, W. (2001). Risk factors associated with *Salmonella enterica* prevalence in three-site swine production systems in North Carolina, USA. *Berliner und Munchener Tierarztliche Wochenschrift*, *114*, 335-338.

Kishima, M., Uchida, I., Namimatsu, T., Osumi, T., & Takahashi, S. (2008). Nationwide surveillance of *Salmonella* in the feces of pigs in Japan. *Zoonoses Public Health*, *55*, 139-144.

Letellier, A., Messier, S., Pare, J., Menard, J., & Quessy, S. (1999). Distribution of *Salmonella* in swine herds in Quebec. *Veterinary Microbiology*, *67*, 299-306.

Lever, M.S., & Williams, A. (1996). Cross-infection of chicks by airborne transmission of *Salmonella enteritidis* PT4. *Journal of Applied Microbiology*, *23*, 347-349.

Mannion, C., Lynch, P.B., Egan, J., & Leonard, F.C. (2007). Efficacy of cleaning and disinfection on pig farms in Ireland. *Veterinary Records*, *161*, 371-375.

Maria, J., Johara, M. Y., Dahlia, H., & Siti Hajar, I. (2002). *Salmonella* serovars isolated in animals and livestock products in Malaysia. *Proceedings of the 12th FAVA and 14th VAM Congress*, 26-28th August 2002. pp. 106.

Mathew, A.G., Cissell, R., & Liamthong, S. (2007). Antibiotic resistance in bacteria associated with food animals: a United States perspective of livestock production. *Foodborne Pathogenic Disease*, *4*(2), 115-13

Nielsen, J. N., Patterson, J. A., Sutton, A., Schinckel, B., Richert, & Boccazzi, P. (1997). The influence of growth promotant antibiotics and management system on the presence and prevalence of *Salmonella* in swine. In *Second International Symposium on Epidemiology and Control of Salmonella in Pork*. Copenhagen, Denmark, 20-22 August, 1997.

Pan, Z.M., Geng, S.Z., Zhu, Y.Q., Liu, Z.Y., Fang, Q., Liu, B.B., & Jiao, X.A. (2010). Prevalence and antimicrobial resistance in *Salmonella* spp. isolated from domestic animals in Eastern China. *Journal of Veterinary and Animal Advances*, *9*(17), 2290-2294.

Paul Schneider. (2002). *Salmonella* control in feeder barns. *Advances in pork production*, *13*, 163.

Rowe, B., Ward, L.R., & Threlfall, E.J. (1997). Multidrug-resistant Salmonella typhi: A worldwide epidemic. *Clinical Infection Disease*, *24*, 106-109.

Schwartz, K.J., Straw, B. E., D'Allaire, S., Mengeling, W.L., & Taylor, D.J. (1999). *Salmonellosis; Diseases of swine*. Oxford: Blackwell Science.

Taylor, J., & McCoy, J.H. (1969). *Salmonella and Arizona infections and intoxications in foodborne infections and intoxications*. New York: Academic Press.

Tran, T.P., Ly, T. L., Nguyen, T. T., Akiba, M., & Ogasawara, N. (2004). Prevalence of *Salmonella* spp.in pigs, chickens and ducks in Mekong Delta, Vietnam. *Journal of Veterinary Medicine and Sciences*, *66*, 1011-1014.

Wondwossen, A., Gebreyes, Siddartha Thakur, & Morgan Morrow, W.E. (2006). Comparison of prevalence, antimicrobial resistance, and occurrence of multidrug-resistant *Salmonella* in antimicrobial-free and conventional pig production. *Journal of Food Protection*, *69*, 67-72.