



## The prevalence and antibiotic resistance of *Salmonella* spp. isolated from pigs and farm environments in Vinh Long province

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### ABSTRACT

The study was conducted to determine the prevalence and antimicrobial resistance of *Salmonella* from pigs and farm environments in Vinh Long province from June 2017 to December 2017. *Salmonella* was isolated from 240 fecal samples and 640 environmental samples (feed, water, barn floor and wastewater) at household and pig farms. There were 76/880 (8.64%) positive samples with *Salmonella*. Among them, *Salmonella* was detected from 35/240 fecal samples (14.58%) which was higher than that from 41/640 environmental samples (6.40%). The prevalence of *Salmonella* in healthy pigs was (8.88%) lower than that of diarrheic pigs (61.54%). The *Salmonella* positive isolation rate from pigs in household farms was 20.20% (62/307) higher than that of pigs in commercial farms with 2.44% (14/573). *Salmonella* positive rate was increased by age; the highest rate was in sow with 37.50% (12/32 samples), following by growing pigs 12.16% (18/148 samples) then post weaning pigs 10.34% (3/29 samples), and lowest rate was in suckling pigs 6.45% (2/31 samples). *Salmonella* isolation from pigs and environments were resistant to trimethoprim/sulfamethoxazole (64.47%), chloramphenicol (52.63%) and ampicillin 46.05%. The multidrug-resistant phenotypes (resisting against 2-7 antibiotics) were observed from 55/76 *Salmonella* isolates, such as Bt - Cl, Am - Bt - Ac and Am - Bt - Cl were the most frequent phenotypes. Besides, these strains were highly sensitive to ceftazidime (100%), levofloxacin (100%), colistin (96.86%), amikacin (97.37%), cefuroxime (97.37%), ofloxacin (96.05%), doxycycline (93.42%), gentamicin (92.11), streptomycin (89.47%), and following to tetracycline (75.00%), amoxicillin/clavulanic acid (75%) and ampicillin (53.95%).

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### 1 INTRODUCTION

*Salmonella* is one of the important foodborne zoonotic pathogens that posed significant public health concerns around the world. It has been the center of microbiological studies for several decades, with

frequent reviews of progress (Gyles *et al.*, 2010, Keelara *et al.*, 2013). It was estimated that approximately one million cases of salmonellosis occurred each year in the United States, causing nearly 20,000 hospitalizations and about 400 deaths (Scallan *et al.*, 2011). It was considered that infected

hosts and environmental factors such as feed, water, barn floor and wastewater were significant sources of *Salmonella* (Lim *et al.*, 2011; Brooks *et al.*, 2014). In Vietnam, *Salmonella* was highly prevalent in pigs (5.2% - 65.4%) (Tran *et al.*, 2004; Tu *et al.*, 2015; Lettini *et al.*, 2016) and livestock environments (8.36%) (Pham Thi Ngoc *et al.*, 2013) Thus, pigs and pig farm environments may play most important reservoirs of human salmonellosis in Vietnam.

Over the years, antibiotics have been used to treat infections in both humans and animals that was effective in control and prevention of diseases. However, it also has created antimicrobial selective pressure leading to form new drug resistant strains (O'Brien, 2002; Garcia-Migura *et al.*, 2014). Increasing resistance or multidrug resistance in Gram-negative bacteria, especially *Enterobacteriaceae*, is a global problem (Partridge, 2010). Multidrug resistant *Salmonella* strains, exhibiting resistance to  $\beta$ -lactams, third generation fluoroquinolones, cephalosporins have been reported in commercial pigs (Aarestrup *et al.*, 2008; Keelara *et al.*, 2013). The resistance of *Salmonella* to several important antibiotics such as ampicillin (58.8%), tetracycline (71%), streptomycin (43.1%), trimethoprim (41.2%) and gentamicin (39.2%) were recorded (Keelara *et al.*, 2013, Lettini *et al.*, 2016). This has caused serious impact on public health by spreading antimicrobial resistant pathogens to human through the food chain or farm environment contamination, either directly or indirectly that led to reduction of the effective treatment and increase of infections on human and animals. This study was conducted to determine the prevalence and antimicrobial resistance of *Salmonella* isolates from pigs and environments in Vinh Long.

## 2 MATERIALS AND METHODS

### 2.1 Materials

A total of 880 samples (240 pig fecal samples and 640 environmental samples) were collected from eight different pig households and farms in Tra On and Tam Binh districts, Vinh Long province from June 2017 to December 2017. Sample size was calculated according to the description of Lwanga and Lemeshow (1991) with 95% confidence interval.

The antimicrobials discs used, include amoxicillin/clavulanic acid (Ac) 20/10  $\mu\text{g}$ , ceftazidime (Cz) 30  $\mu\text{g}$ , cefuroxime (Cu) 30  $\mu\text{g}$ , colistin (Co) 10  $\mu\text{g}$ , gentamicin (Ge) 10  $\mu\text{g}$ , amikacin (Ak) 30  $\mu\text{g}$ , streptomycin (Sm) 10  $\mu\text{g}$ , tetracycline (Te) 30  $\mu\text{g}$ , doxycycline (Dx) 30  $\mu\text{g}$ , chloramphenicol (Cl) 30  $\mu\text{g}$ , bactrim (Bt) 1,25/23,75  $\mu\text{g}$ , ampicillin (Am) 10  $\mu\text{g}$ , ofloxacin (Of) 5  $\mu\text{g}$  and levofloxacin (Lv) 5  $\mu\text{g}$  (Nam Khoa Co., Ltd., Vietnam).

## 2.2 Methods

### 2.2.1 Sample collection

**Fecal samples:** Fecal samples (approximately 25 g) were aseptically collected with gloved hands after they were released into the environment from healthy and diarrheic pigs at all ages. At each farm, 3 – 4 barns were selected randomly. From each selected barn, 3 – 5 fecal samples were collected. Samples were collected every four weeks. Collected samples were placed in sterile plastic bags with information and stored at cold condition.

**Environmental samples:** Feed (250 g), drinking water (1,000 ml), wastewater (1,000 ml) and barn floor swab were randomly collected at every stage of fecal sampling in the same barn. Feed samples were collected per each barn at three sites including feed in bag, feeder bin, and leftovers in the trough. Drinking water samples were taken from the source of water, drinking nipple or drinking trough. Barn floor swab samples were aseptically collected using cotton swabs at two different sites in the same pen. Wastewater was collected at wastewater draining from the barns and the end of wastewater flow from the reservoirs before flowing to the rivers.

### 2.2.2 Isolation, identification and antimicrobial resistance testing of *Salmonella*

Isolation and identification of *Salmonella* were performed following the description of Barrow and Feltham (2003) and National standards of Vietnam TCVN 4829:2005 (Ministry of Science and Technology, 2016). All the *Salmonella* isolates from pigs and environments were tested against to 14 antimicrobials using disc diffusion method (Bauer *et al.*, 1966). The diameter of inhibition zones surrounding the antimicrobial discs was interpreted in accordance to the guidelines of the Clinical Laboratory Standards Institute (CLSI) in 2016.

### 2.2.3 Statistical analysis

The data were analyzed by Chi-square test using Minitab 16.0 software for value more than 5; Yates's chi-squared test using Microsoft Excel 2010 software when at least one cell of the table has an expected count  $\geq 2$  and  $\leq 5$ .

## 3 RESULTS AND DISCUSSIONS

### 3.1 The result of *Salmonella* isolation in pigs and the environmental farms

Overall, the presence of *Salmonella* in pigs and the farm environment was quite high. The prevalence of *Salmonella* positive samples in the feces was 14.58% (35/240 samples), which was higher than that of the environmental samples with 6.40% (41/640 samples) ( $p=0.00$ ). Many studies over the

past 10 years in several provinces of the Mekong Delta and Central Vietnam, the results showed that *Salmonella* prevalence was significantly high in pigs from 5.2% to 65.4% (Tran *et al.*, 2004; Tu *et al.*, 2015; Lettini *et al.*, 2016) which were consistent with this present study. The high prevalence of *Salmonella* in pigs might be due to nutritional deficiency from reducing the amount of feed diets, and poor nursing and feeding process in pigs. According to Pires *et al.* (2014), besides the number of bacteria and individual health status, the stress from reduced feed was also as a factor that made to increase of

microorganism numbers and pig infections. Although *Salmonella* was a lower proportion (6.40%) in environmental farms, they play as a potential source of *Salmonella* infection from healthy pigs to diarrheic pigs, from farms to farms, or/and regions to regions. The reports from different countries such as South Korea and the United States indicated that the prevalence of *Salmonella* in feces was higher or lower than that in environments which depended on the surveyed farms and livestock characteristics. (Lim *et al.*, 2011, Keelara *et al.*, 2013; Pires *et al.*, 2013).

**Table 1: The prevalence of *Salmonella* in pigs and environmental farms in Vinh Long province**

Samples	No. of examined samples	No. of positive samples	Percentage (%)
Feces	240	35	14.58
Environment	640	41	6.40
			<i>P</i> = 0.00
Total	880	76	8.64

**Table 2: The prevalence of *Salmonella* in pigs and environmental farms by farm types**

Type	No. of examined sampl	No. of positive samples	Percentage (%)
Household	307	62	20.20
Farm	573	14	2.44
			<i>P</i> = 0.00
Total	880	76	8.64

The proportion of *Salmonella* positive in households (20.20%) was higher than that in farms (2.44%) (*p*=0.00). Rasschaert *et al.* (2012) suggested that type of farms influenced the prevalence of *Salmonella* on farms. In addition, prophylactic factors and disinfection play an important role in *Salmonella* control (Pires *et al.*, 2014). *Salmonella* contamination could be derived from poor cleaning and disinfection, contaminated feed and water systems. In household farms with a small herd size (6-40 pigs), a traditional practice in simple disease prevention process, vaccination and sanitation were not focused. Furthermore, the inadequate wastewater

treatment system, and farmers did not provide knowledge of animal husbandry, that may cause increase of *Salmonella* prevalence in pigs and environmental farms. For pig farms with herd size over 100 pigs, hygiene and disease prevention procedures were implemented strictly and effectively. Besides, feed and drinking water sources from these farms were also treated before using for pigs. As a result, infection of pathogens to pigs and their habitats were decreased.

*Salmonella* was isolated from 204 healthy pigs and 36 diarrheic pigs, the result was shown in Table 3.

**Table 3: The prevalence of *Salmonella* in healthy and diarrheic pigs in Vinh Long province**

Samples	No. of examined samples	No. of positive samples	Percentage (%)
Healthy pig	204	19	9.31
Diarrheic pig	36	16	44.44
			<i>P</i> =0.00
Total	240	35	14.58

The prevalence of *Salmonella* in diarrhea pigs was 44.44% (16/36 samples) higher than that in healthy pigs with 9.31% (19/204 samples), and there was statistically significant difference (*p*=0.00). *Salmonella* is a member of *Enterobacteriaceae* (Gyles *et al.*, 2010). They can overcome colonization resistance of the gut microbiota and exist in the intestinal tract of human and animals (Ahmer and

Gunn, 2011). Some evidences suggested that *Salmonella* was excreted in feces of both diseased and carrier pigs without clinical signs (De Jong *et al.*, 2014; Jiu *et al.*, 2017). Especially, pathogens were shed with large numbers from diseased pigs. In addition, for diarrhea pigs, fecal samples were collected selectively from pigs with specific clinical manifestations of salmonellosis such as febrile with temper-

atures of 105–107°F (40.5–41.6°C), constipation initially following by watery yellow feces diarrhea or coldness at the top of ears (Carlson *et al.*, 2012). Thus, *Salmonella* positive proportion of diarrhea

pigs was high in this study that could explain. This result suggested that diseased pigs are the main source of *Salmonella* excretion into the environmental farms in Vinh Long province.

**Table 4: The prevalence of *Salmonella* in pigs and environmental farms by pig stages**

Pig stage	No. of examined samples	No. of positive Samples	Percentage (%)
Piglet	31	2	6.45
Weaning	29	3	10.34
Growing-finishing	148	18	12.16
Sow	32	12	37.50
<i>P</i> ( <i>H</i> <sub>0</sub> )=0.003			
Total	240	35	14.58

Overall, the highest excretion of *Salmonella* was observed among sow group (37.50%) followed by growing-finishing pigs (12.16%), weaning (10.34%) and piglets (6.45%) with *P* value= 0.003. The low frequency of *Salmonella* in piglets was presumably result of pathogenic resistance by colostrum’s immunity. Weaning pigs can infect *Salmonella* by the fecal - oral route and develop a disease as the same rate to piglets (Carlson *et al.*, 2012). *Salmonella* isolation were a high prevalence

on sows because they live a long time in the farms and were infected by pathogen reservoirs in the environment (Funk and Gebreyes, 2004). This study suggested that sows were a carrier and as an important source of *Salmonella* shedding to the environment on the pig farms in Vinh Long province. The exposure of *Salmonella* in this study was appropriate with Lynch *et al.* (2018) who reported that *Salmonella* was isolated with highest rate in sows (5.00%) and lowest rate in piglets (4.00%).

**Table 5: The prevalence of *Salmonella* in environmental farms in Vinh Long province**

Samples	No. of examined samples	No. of positive samples	Percentage (%)
Barn floor	118	16	11.02
Wastewater	95	6	6.32
Feed	258	13	5.04
Drinking water	169	6	3.55
<i>P</i> =0.00			
Total	640	41	6.40

*Salmonella* spp. were distributed in not only animals but also in various environments such as farmhouses and vectors (Barber *et al.*, 2002). The proportion of positive environmental samples were rather high from 3.55% to 11.02%. *Salmonella* strains were isolated from various environmental samples, and the highest prevalence was observed in barn floors (11.02%), followed by wastewater (6.32%), feed (5.04%), and drinking water (3.55%) (*P*=0.00). *Salmonella* was present with a quite high level (11.02%) at barn floors that might be due to direct contact with feces excreted from swine frequently. Although some of these samples were collected from the dry barn floors, without fecal contamination, *Salmonella* was still detected, indicating that the barn floor was considered as a source of pathogenic contamination at the farms. A ratio of 5.04% (13/258) of feed samples was *Salmonella* positive, similarly to previous researches of Molla *et al.*, (2010); Lynch *et al.*, (2018) in United States and Iceland. Additionally, *Salmonella* was also found in 6 of 169 (3.55%) drinking water samples, taking from the source of water, drinking nipples and

drinking trough. Detection of *Salmonella* in feeds which were collected from feeder bins and troughs and drinking water of swines may result in the contamination of the bacteria from swines or barn floors at the same pen. Hanes (2003) considered that *Salmonella* can survive under different environmental conditions. They can exist for a long time in the environment after releasing from feces of carriers, particularly in swine manure wastewater of farms. In this study, out of the total number of wastewater samples examined, *Salmonella* was detected in 6.32% (6/95), and previous investigations reported wastewater as reservoir of *Salmonella* at swine farms (Brooks *et al.*, 2014, Huong *et al.*, 2014). *Salmonella* contaminated water source could be due to untreated wastewater containing pathogens flow to the river or canals near farms. These results suggest environment contaminated with *Salmonella* could pose an infection risk to swine on swine farms.

### 3.2 Antimicrobial resistance of *Salmonella* isolated from pigs and environmental farms

*Salmonella* isolates were resistant to trimethoprim/sulfamethoxazole (64.47%), chloramphenicol (52.63%) and ampicillin (46.05% %). They were low resistant to amoxicillin/clavulanic acid (25.00%), tetracycline (25.00%), streptomycin (10.53%), gentamicin (7.89%) and doxycycline (6.58%). However, the isolates showed no resistance to ceftazidime and levofloxacin. According to Garcia-Migura *et al.* (2014), the emergence of antibiotic resistance strains has been associated with the consumption of antimicrobials in veterinary medicine. Through the survey, trimethoprim/sulfamethoxazole, chloramphenicol and ampicillin were commonly used to prevent and treat some gastrointestinal diseases in swines and as growth promoters at study areas. Therefore, it could be the reason for resistance of *Salmonella* to 12/14

antibiotics, among them, there were 4/12 strains which were resistant less than 6% (1.32%-3.95%). Tadee *et al.* (2014) tested 200 *Salmonella* isolates from fecal pigs and the environment at the pig farms in northern Thailand. The results showed that most strains were resistant to ampicillin (81.5%), tetracycline (74.5%) and streptomycin (70.5%). However, the strains were susceptible to amoxicillin / clavulanic acid (100.00%), trimethoprim / sulfamethoxazole (64.5%) and chloramphenicol (78.5%). The differences about geography, characteristics of isolates and antibiotics using in the study sites may cause differences in the antibiotic resistance characteristics of *Salmonella*. *Salmonella* isolates in Vinh Long province were still susceptible to several antimicrobials. This may be due to the significant reduction of antibiotics use in order to save livestock production expenses when pig prices decline. Bacteria did not expose to antimicrobials; therefore, resistant strains were not formed.

**Table 6: Antimicrobial resistance of *Salmonella* isolated from pigs and environmental farms (n=76)**

Group	Antimicrobial group	Antimicrobial	Resistance		Sensitive	
			Number	(%)	Number	(%)
N1	β-lactam	Am	35	46.05	41	53.95
		Ac	19	25.00	57	75.00
		Cz	0	0.00	76	100.00
		Cu	2	2.63	74	97.37
N2	Polypeptides	Co	1	1.32	75	98.68
N3	Aminoglycosides	Ge	6	7.89	70	92.11
		Ak	2	2.63	74	97.37
		Sm	8	10.53	68	89.47
	Tetracyclines	Te	19	25.00	57	75.00
		Dx	5	6.58	71	93.42
		Cl	40	52.63	36	47.37
N4	Phenicol	Bt	49	64.47	27	35.53
		Lv	0	0.00	76	100.00
		Of	3	3.95	73	96.05

The distribution of multidrug resistance patterns was summarized in Table 7.

There were 55 *Salmonella* strains among the 76 isolates that showed resistant to 2-7 antibiotics, with 27 different resistance patterns. In particular, multi-drug-resistant phenotype with many *Salmonella* strains concentrated in two and three antibiotics with 34.55% (19/55 isolates) and 27.27% (15/55 isolates), following by four antibiotics with 7.27%

(4/55 isolates), sixantibiotics with 5.45% (3/55) and seven antibiotics with 3.64% (2/55 isolates). Among them, the most frequent resistance phenotypes observation were Bt – Cl (23.64%), Am - Bt – Ac (10.91%) and Am - Bt – Cl (10.91%). The report of Tamang *et al.* (2015) in Korea demonstrated that *Salmonella* isolates from pigs were resistant to several antimicrobials such as ampicillin, gentamicin, tetracycline, and that was the most common resistant pattern.

**Table 7: Multi-antimicrobial resistance of *Salmonella* isolated from pigs and environmental farms (n=55)**

No. of antimicrobial resistance	No. of multidrug-resistant phenotype	Multidrug-resistant phenotype	No. of isolates	Percentage (%)		
2	6	Cl+Am	2	3.64		
		Cl+Bt	13	23.64		
		Am+Sm	1	1.82		
		Te+Sm	1	1.82		
		Te+Bt	1	1.82		
		Cu+Of	1	1.82		
3	3	Te+Cl+Bt	3	5.45		
		Am+Bt+Ac	6	10.91		
		Cl+Am+Bt	6	10.91		
4	9	Cl+Am+Bt+Ac	1	1.82		
		Te+Am+Bt+Ac	3	5.45		
		Te+Cl+Dx+Bt	1	1.82		
		Te+Cl+Am+Bt	1	1.82		
		Ak+Cl+Am+Bt	1	1.82		
		Cl+Ge+Am+Sm	1	1.82		
		Cl+Dx+Am+Bt	1	1.82		
		Cl+Am+Bt+Sm	2	3.64		
		Ge+Am+Bt+Ac	1	1.82		
		5	4	Ge+Of+Am+Bt+Ac	1	1.82
				Te+Cl+Am+Bt+Ac	1	1.82
Te+Dx+Am+Bt+Ac	1			1.82		
Cl+Co+Am+Bt+Ac	1			1.82		
6	3	Te+Cl+Ge+Am+Bt+Sm	1	1.82		
		Te+Ak+Cl+Dx+Bt+Ac	1	1.82		
		Te+Cl+Ge+Am+Bt+Ac	1	1.82		
7	2	Te+Cl+Ge+Dx+Am+Bt+Sm	1	1.82		
		Te+Cl+Cu+Of+Am+Bt+Sm	1	1.82		
Total	27		55	100.00		

As a result, the antibiotic resistance of *Salmonella* in swine has tended to increase about number of antibiotics and proportion in Vinh Long province. They were not only resistant to many antibiotics, but they also carried a diversified and complicated resistant phenotypes. Multidrug resistance was caused by the accumulation of natural and the acquired resistance as a result of the acquisition or alteration of genes that regulate resistance. Therefore, bacteria become susceptible to few antibiotics and resistant to several antimicrobial agents or antimicrobial groups (Partridge, 2010; Macgowan and Macnaughton, 2017)

#### 4 CONCLUSIONS

The prevalence of *Salmonella* in pigs and environmental farms in Vinh Long province was at high rate that depends on farm types. *Salmonella* is one of the causes of diarrhea on pigs and present regularly in healthy pigs. Sows were carriers and important sources of *Salmonella* shedding to the environment of pig farms. Farm environment factors were as the potential vehicles of *Salmonella* transmission.

*Salmonella* isolates in Vinh Long province were resistant several antimicrobials in clinical practice such as ampicillin, trimethoprim/sulfamethoxazole and chloramphenicol. They expressed multi-resistant antibiotics characteristics with diversified and complicated phenotypes. These strains were still sensitive to ceftazidime, levofloxacin, colistin, amikacin, cefuroxime, ofloxacin, doxycycline, gentamicin, amoxicillin/clavulanic acid, streptomycin and tetracycline which could be used to prevent and treat salmonellosis in pigs in Vinh Long province.

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