Journal of Agriculture and Technology Management (JATM)
21(1): 1-7 (January-June 2018)

Original article

High prevalence of Antibiotic Resistance in *Salmonella* and *Escherichia coli* isolated from Pig farms and Slaughterhouses in North Vietnam

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ABSTRACT

This study presents the high prevalence of antibiotic resistance of *Salmonella* and *E. coli* among selected pig farms and slaughterhouses in three provinces and two cities in North Vietnam. *Salmonella* and *E. coli* were mostly isolated from the sewage, anus feces, caecum, slaughterhouse's floors, animal keeping floors, piggery floors, and carcass samples. The prevalence of *Salmonella* was 14.0% and 40.9%, and meanwhile, *E. coli* were 68.2% and 67.2% of the collected samples at the pig farms and slaughterhouses, respectively. Among the 189 *Salmonella* isolates, 179 isolates were serotypeable belong to 10 serovars. Derby (21.7%) was the most common serovar, followed by Typhimurium (18.5%), Anatum (14.3%), Rissen (12.2%) and London (8.5%). The other serovars were Agona, Weltevreden, Meleagridis, Braenderup, and Chartres. Resistance to streptomycin (81.5% and 78.0%), tetracycline (78.3% and 89.0%), and ampicillin (55.6% and 64.0%) were most commonly observed in *Salmonella* and *E. coli*, respectively. Markedly lower resistance rates were observed in both of these bacteria for trimethoprim, nalidixic acid, ceftazidime, norfloxacin, nitrofurantoin, ciprofloxacin, and gentamicin. Interestingly, decreased susceptibility to quinolones group (ciprofloxacin, norfloxacin, and nalidixic acid) and third-generation cephalosporins (ceftazidime) was commonly detected in the *Salmonella* and *E. coli* isolates. Moreover, resistance rates of the *E. coli* isolate to trimethoprim, norfloxacin, nalidixic acid, ciprofloxacin, and gentamicin were significantly higher than those in the *Salmonella* isolates ($p<0.05$).

KEYWORDS: *antibiotic resistance, E. coli, Salmonella, farms, North Vietnam, slaughterhouse*

1 INTRODUCTION

Antibiotics have played an indispensable role in the treatment of infectious diseases in animals and humans. However, selective pressure exerted by antibiotics use also has been the major driving force behind the emergence and spread of drug-resistance among pathogenic and commensal bacteria (Aarestrup *et al.*, 2008). Clones of multi-drug resistant bacteria have globally disseminated, and in some cases, infections are essentially untreatable with existing antimicrobial agents. Assessing the implications of increasing prevalences of antimicrobial resistant pathogens is important for defining the prognosis for the individual case with infection and knowledge about the outcome of infections with resistant organisms gives physicians and hospitals an impetus to use good infection and antibiotic controls to prevent such infections. Finally, understanding the effect of antibiotic resistance on patient outcomes is relevant for policymakers, who must make decisions about funding of programs to track and prevent the spread of antimicrobial-resistant organisms.

Due to contamination, antibiotic-resistant bacteria can get to the food products, for example via smear infections with fecal bacteria, polluted spray water or due to poor production hygiene (Schegelova *et al.*, 2004). Food contamination with antibiotic-resistant bacteria can be a major threat to public health, as the antibiotic resistance determinants can be transferred to other bacteria of human clinical significance. In fact, over the last few years, several studies have documented the emergence of antibiotic resistance in the bacterial foodborne pathogens, especially, in *Enterobacteriaceae* family (Lynch *et al.*, 2013; Livermore, 2013). In the *Enterobacteriaceae* family, *Salmonella* and *E. coli* are the major bacterial foodborne pathogens. These bacteria are reported to be resistant with many antibiotics at the high rates, and rapidly increasing worldwide (Mayrhofer *et al.*, 2004; Gousia *et al.*, 2011; Koluman and Dikici, 2013).

The excessive use of antibiotics in Vietnam may partially be responsible for the high incidence of the antibiotic-resistant bacteria. It was previously reported that antibiotic-resistant bacteria were more frequently isolated in both human (Le *et al.*, 2009; Dyar *et al.*, 2012; Nguyen *et al.*, 2013) and animals (Van *et al.*, 2007a; Van *et al.*, 2007b; Vo *et al.*, 2010; Thai *et al.*,

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Received: 21 May 2018; Accepted: 14 June 2018
p-ISSN: 2599-4875 e-ISSN: 2599-4980

2012) in Vietnam. Since pathogenic foodborne bacteria generally become resistant to antibiotics in the digestive tract of host animals, it is also important to understand the reservoir of these antibiotic resistance among intestinal bacteria and how gut bacteria in food animals are involved in the dissemination of antibiotic resistance.

Escherichia coli (E. coli) and *Salmonella* are primary intestinal commensal organisms found in endothermic animals and widely disseminated in the environment. Both of these bacteria cause zoonosis in human and animals. Unlike *E. coli*, *Salmonella* spp. is recognized as important primary zoonotic pathogens. Investigating potential pathogenic *E. coli* strains and/or antibiotic resistance may provide information regarding human activity in select ecological niches. It indicated that living in proximity to humans may alter the *E. coli* populations of wildlife intestinal microbiota, which can, in turn, serve as a reservoir of *E. coli* strains that are pathogenic to humans. However, to our knowledge, there have never been intensive studies made on prevalence and antibiotic resistance dissemination among *Salmonella* and *E. coli* isolated in pig farms and slaughterhouses in Vietnam. This study was conducted to address some of these issues and to provide current information on prevalence, antibiotic resistance of *Salmonella* and *E. coli* among selected pig production farms and slaughterhouses in North Vietnam.

2 MATERIALS AND METHODS

Sample collection and bacterial isolation

From January 2009 to December 2010, 315 samples from 15 pigs farms (in three provinces Bac Ninh, Hai Duong, Vinh Phuc; and two cities Ha Noi, Hai Phong at the north Vietnam) including anus feces (n=120), piggery floors (n=60), drinking waters (n=45), animals foods (n=40), sewage (n=30) and tools (n=20); and 330 samples from two related slaughterhouses (in Ha Noi city) including carcass (n=90), caecum (n=90), anus feces (n=77), keeping animal floors (n=40), slaughterhouse's floor (n=20) and water tanks (n=13) were collected. Swab samples (anus feces, floors, tools, and carcass) were collected by autoclaved cotton in an area approximately 20 cm2 and placed in sterile bags with 90 mL of buffered peptone water (BPW). Approximately 100 mL of drinking waters, water tanks, and sewage were taken to 200 mL bottle and then 10 mL was mixed with 90 mL of BPW. Caecum and food samples were taken to the sterilized plastic bags and transported to our laboratory. At the laboratory, 1 gram of the substance from caecum; 25 gram of food samples were homologized with BPW following the ratio 1:10 and incubated at 370C within 18 - 24h for pre-enrichment. All samples were kept on ice box during the transportation and characterized on the arrival day at the laboratory of the Department of Veterinary Hygiene, National of Institute Veterinary Research, Vietnam. The next steps for *Salmonella* isolation were previously described (Thai *et al.*, 2012). For *E. coli* isolation, 0.1 mL of the ratio 1:10 pre-enrichment cultures were continuously incubated onto eosin methylene blue agar (EMB) at 37°C for 24 h. Only one typical colony producing the metallic sheen on EMB agar were isolated and streaked into the triple sugar iron agar (TSI) tube and incubated at 37°C for 24 h. The colonies in TSI agar showed typical *E. coli* characteristics, such as glucose and lactose fermentation with gas production and the absence of H2S, were confirmed by gram staining and a biochemical series based on citrate utilization, indole production, methyl red and Voges-Proskauer reactions.

Salmonella serotyping

The typical *Salmonella* isolates were serotyped on slides by the microtiter agglutination test to identify O and H antigens (Difco Laboratories, Detroit, MI, USA), according to the version of the Kauffmann and White scheme (Grimont and Weill, 2007).

Antibiotic susceptibility testing

In this study, the 189 *Salmonella* and 200 *Escherichia coli* isolates were selected to characterize the antibiotic susceptibility. The antibiotic susceptibility of isolates was determined according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2006). Agar diffusion assays were performed on Muller - Hinton agar with disks containing 10 different antibiotic agents (Oxoid, UK). The tested antibiotics were as follows: ampicillin, 10 µg; ceftazidime, 30 µg; ciprofloxacin, 5 µg; gentamicin, 10 µg; nalidixic acid, 30 µg; norfloxacin, 10 µg; streptomycin, 10 µg; tetracycline, 30 µg; trimethoprim, 5 µg and nitrofurantoin, 300 µg. The susceptible, intermediate or resistant interpretive categories were used according to CLSI guidelines (CLSI, 2010). *Escherichia coli* ATCC 25922 was used as the quality control organisms.

Data and statistical analysis

Statistical comparison of the prevalence, antibiotic resistance rates between *Salmonella* and *Escherichia coli* from different sources were analyzed by the Chi-square test.

3 RESULTS AND DISCUSSION

The prevalence of *Salmonella* and *E. coli*

The table 1 and table 2 presented the prevalence of *Salmonella* and *E. coli* at the pig farms and slaughterhouse.

For *Salmonella* isolation, the bacterium was isolated from 44 (14.0%) out of 315 samples and 135 (40.9%) out of 330 samples collected from the pig farms and slaughterhouses, respectively. At the farms, *Salmonella* presented with the rates 25.0% and 21.7% in anus feces and piggery floors, respectively. At the slaughterhouses, *Salmonella* was highly isolated from the anus feces (55.5%), caecum (47.7%), slaughterhouse's floors (40.0%), animal keeping floors (37.5%), and carcass (30.0%). Interestingly, the tools, food, sewage and water tanks samples were not conta-

p-ISSN: 2599-4875 e-ISSN: 2599-4980

minated with *Salmonella*.

For *E. coli* isolation at the farm level, the bacterium was isolated from 215 (68.2%) out of 315 samples. Of which, 30 (100%) sewage, 113 (94.2%) anus feces, 56 (93.3%) piggery floors, 13 (28.9%) drinking water, and 3 (15.0%) tools samples were positive for *E. coli*. At the slaughterhouses, the highest isolation rate of *E. coli* was at slaughterhouse's floors (90.0%), followed by caecum samples (83.3%), anus feces (79.2%), animal keeping floors (77.5%), and carcass samples (24.4%).

Except for the carcass samples, the isolation rates of *E. coli* in the positive samples were always significantly higher than those of *Salmonella* in both farms and slaughterhouses (P<0.05). There was no significant difference between the prevalence of *E. coli* at the farms and slaughterhouses. However, the prevalence of *Salmonella* at slaughterhouses was significantly higher than that at the farms (p<0.05).

Salmonella serotyping

Among the *Salmonella* isolates, 179 isolates were serotypeable belong to 10 serovars and 10 isolates were un-serotypeable (Table 3). Derby (21.7%) was the most common serovar, followed by Typhimurium (18.5%), Anatum (14.3%), Rissen (12.2%), and London (8.5%). The prevalence of serovars Chartres, Braenderup, Meleagridis, Weltevreden, and Agona ranged from 1.6% to 5.8%.

Antibiotic resistance of *Salmonella* and *E. coli*

Tables 4 and 5 showed the prevalence of antibiotic resistance observed in the *Salmonella* and *E. coli* isolates.

For the *Salmonella* isolates, resistance to streptomycin (81.5%), tetracycline (78.3%), and ampicillin (55.6%) were most often observed. Markedly lower resistance rates were observed for trimethoprim (25.4%), nitrofurantoin (21.2%), nalidixic acid (19.6%), ceftazidime (17.5%), norfloxacin (15.9%), ciprofloxacin (13.8%), and gentamicin (11.1%).

For the 200 selected *E. coli* isolates, the most frequently observed resistance in *E. coli* was to tetracycline (89.0%), followed by resistance to streptomycin (78.0%), ampicillin (64.0%), trimethoprim (61.5%), and nalidixic acid (56.5%). Resistance to gentamicin, nitrofurantoin, ciprofloxacin, and norfloxacin ranged from 21.0% to 38.5%. Although, only 15 (7.5%) *E. coli* isolates were observed to be resistant with ceftazidime, more than half of them displayed the decreasing susceptibility to this antibiotic.

Interestingly, decreased susceptibility to quinolones group (ciprofloxacin, norfloxacin, and nalidixic acid) and third-generation cephalosporins (ceftazidime) were commonly observed in the *Salmonella* and *E. coli* isolates. Moreover, resistance rates of the *E. coli* isolate to trimethoprim, norfloxacin, nalidixic acid, ciprofloxacin, and gentamicin were significantly higher than those in the *Salmonella* isolates (p<0.05).

Salmonella and *E. coli* species are ubiquitous in the environment and can colonize and cause disease in a variety of food producing and non-food producing animals. This study presents the high prevalence of *Salmonella* and *E. coli* at the pig farms and, especially, at the slaughterhouses, it may explain for the high contamination rates of these bacteria in the raw meat samples in previous reports in Vietnam (Van *et al.*, 2007a; Van *et al.*, 2007b; Thai *et al.*, 2012). This situation reflected the poor management and hygiene condition in both pig farms and slaughterhouses in Vietnam. Among the *Salmonella* isolates, 10 serovars were identified including Derby, Typhimurium, Anatum, Rissen, London, Agona, Weltevreden, Meleagridis, Braenderup, and Chartres. In overall, most of these serovars were commonly detected in animals, and food products in previous research in Vietnam (Vo *et al.*, 2006; Van *et al.*, 2007a; Thai *et al.*, 2012) and other Asian countries (Bangtrakulnonth *et al.*, 2004; Galanis *et al.*, 2006). Interestingly, two serovars Chartres, Meleagridis which more commonly detected in Europe, the US and China (Ebner *et al.*, 2004; Wales *et al.*, 2009; Li *et al.*, 2014) and have not been reported in Vietnam, also detected in this study. Moreover, the high prevalence of serovars Typhimurium in the present study is alarming for the food safety and public health in Vietnam.

Salmonella and *E. coli* are not only a public health concern due to the number of cases per year, but many strains are resistant to several antibiotics. The high resistance rates of *Salmonella* and *E. coli* to tetracycline, streptomycin, and ampicillin were previously reported from Vietnam (Van *et al.*, 2007a; Van *et al.*, 2008; Vo *et al.*, 2010; Thai *et al.*, 2012). However, in our research, the *Salmonella* and *E. coli* isolates displayed the very high rates of resistance to tetracycline (78.3% and 89.0%), streptomycin (81.5% and 78.0%), and ampicillin (55.6% and 64.0%), respectively. The reason may due to the different collection sources of the bacteria isolates between the previous reports above and this present study. *Salmonella* and *E. coli* isolates in the present study were recovered from the farms and related slaughterhouse where they were usually exposed to these antibiotics which were extensively used in livestock production (Araque, 2009; Gousia *et al.*, 2011; Kaesbohrer *et al.*, 2012). Resistance to the quinolones group (ciprofloxacin, 13.8%; norfloxacin, 15.9%; and nalidixic acid, 19.6%) of the *Salmonella* isolates was higher than those at the previous reports in Vietnam (Van *et al.*, 2007a; Van *et al.*, 2007b; Thai *et al.*, 2012) and other countries (Clemente *et al.*, 2013; Jones Dias *et al.*, 2013; de Jong *et al.*, 2014). Although, quinolone-resistant *E. coli* were reported worldwide (Huotari *et al.*, 2003; Van *et al.*, 2008; Wasyl *et al.*, 2013). However, the high resistance rates of the *E. coli* isolates to ciprofloxacin (24.5%), norfloxacin (38.5%) and nalidixic acid (56.5%) do not only reflect the lenient use of this antibiotic among farms, but also alarm the public health concern in Vietnam because of the spread of antibiotic resistance from farm-animal bacteria to human bacteria in the human digestive tract was confirmed (Hammerum and Heuer, 2009; Hunter *et al.*, 2010; Jones Dias *et al.*, 2013); and these drugs are the most valuable antibiotics for treatment of human

p-ISSN: 2599-4875 e-ISSN: 2599-4980

bacterial infections. Likewise, the strict regulation would be executed to curb the use of these antibiotics, especially, in the animal production chains in Vietnam. Recently, some reports have already described the reduced susceptibility to the third generation cephalosporins (ceftazidime) in *Salmonella* and *E. coli* strains from food products, veterinary and human sources around the world (Bouchrif *et al.*, 2009; Shaheen *et al.*, 2011; Clemente *et al.*, 2013). However, resistance to this antibiotic in *Salmonella* and *E. coli* has not been reported in Vietnam (Van *et al.*, 2007a; Van *et al.*, 2008; Vo *et al.*, 2010; Thai *et al.*, 2012). Notably, the high resistance rates of the *Salmonella* (7.5%) and *E. coli* (15.7%) isolates to ceftazidime were commonly observed in this study; hence it may create the serious problem for the treatment of pathogenic bacteria infection in both human and veterinary sides in Vietnam.

Overall, this data showed that good hygiene, strict biosecurity measures from farms to table should be practiced in Vietnam. Moreover, the usages of alternative growth promotants, use of probiotics are good to decrease the use of antimicrobials in livestock.

4 CONCLUSION

In summary, this study showed a high prevalence of *Salmonella* and *E. coli* at the pig farms and related slaughterhouses. The antibiotic resistance rates of the Salmonella and E. coli isolates were much higher than those in other previous reports in Vietnam. This situation poses a risk because of the transmission of antibiotic-resistant foodborne pathogens to humans through the food chain and leads to clinical antibiotic chemotherapy failure. Therefore, it is necessary to plan intervention strategies to appropriately manage the use of antibiotics in the livestock industry and to control the spread of these pathogens for the public health in Vietnam.

ACKNOWLEDGMENT

This study was supported by the Ministry of Agriculture and Rural Development, Vietnam for two years, from 2009 to 2010. The authors would like to thank the cooperators at the farms and slaughterhouses, students, technicians, and other individuals who helped us in sampling and processing.

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p-ISSN: 2599-4875 e-ISSN: 2599-4980

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Table 1. The prevalence of *Salmonella* and *E. coli* at the pig farms

Type of samples	Samples (no.)	No. positive samples (%) for	
		Salmonella	E. *coli*
Anus Feces	120	30 (25.0)	113 (94.2)
Piggery floor	60	13 (21.7)	56 (93.3)
Tools	20	0 (0.0)	3 (15.0)
Commercial Pig Feed	40	0 (0.0)	0 (0.0)
Drinking water	45	1 (2.2)	13 (28.9)
Sewage	30	0 (0.0)	30 (100)
Total	**315**	**44 (14.0)**	**215 (68.2)**

Table 2. The prevalence of *Salmonella* and *E. coli* at the pig slaughterhouses

Type of samples	Samples (no.)	No. positive samples (%) for	
		Salmonella	E. *coli*
Carcass	90	27 (30.0)	22 (24.4)
Caecum	90	42 (47.7)	75 (83.3)
Water tanks	13	0 (0.0)	0 (0.0)
Animal keeping floor	40	15 (37.5)	31 (77.5)
Anus swab	77	43 (55.8)	61 (79.2)
Slaughterhouse flooring	20	8 (40.0)	18 (90.0)
Total	**330**	**135 (40.9)**	**207 (62.7)**

Table 3. Serovars identification for the *Salmonella* isolates from the pig farms and slaughterhouses

Order	Serovars	Number (%)
1	Anatum	27 (14.3)
2	Agona	11 (5.8)
3	Braenderup	4 (2.1)
4	Chartres	3 (1.6)
5	Derby	41 (21.7)
6	London	16 (8.5)
7	Meleagridis	9 (4.8)
8	Rissen	23 (12.2)
9	Typhimurium	35 (18.5)
10	Weltevreden	10 (5.3)
11	Un-serotypeable	10 (5.3)
	Total	**189 (100)**

Table 4. The prevalence of antibiotic resistance of the Salmonella isolates from the pig farms and slaughterhouses

Antibiotics	No. of isolates	Antibiotic susceptibility of the *Salmonella* isolates		
		Susceptible no. (%)	Intermediate no. (%)	Resistant no. (%)
Ampicillin	189	38 (20.1)	46 (24.3)	105 (55.6)
Ciprofloxacin	189	131 (69.3)	32 (16.9)	26 (13.8)
Ceftazidime	153 (81.0)	115 (60.8)	41 (21.7)	33 (17.5)
Gentamicin	80 (42.3)		15 (7.9)	21 (11.1)
Nalidixic acid	189		72 (38.1)	37 (19.6)
Nitrofurantoin	189	125 (66.1)	24 (12.7)	40 (21.2)
Norfloxacin	189	133 (70.4)	26 (13.8)	30 (15.9)
Streptomycin	189	20 (10.6)	25 (13.2)	154 (81.5)
Trimethoprim	189	93 (49.2)	38 (20.1)	48 (25.4)
Tetracycline	189	15 (7.9)	26 (13.8)	148 (78.3)

p-ISSN: 2599-4875 e-ISSN: 2599-4980

Table 5. The prevalence of antibiotic resistance of the 200 selected E. coli isolates from the pig farms and slaughterhouses

Antibiotics	**No. of isolates**	**Antibiotic susceptibility of the E. *coli* isolates**		
		Susceptible no. (%)	**Intermediate no. (%)**	**Resistant no. (%)**
Ampicillin	200	12 (6.0)	60 (30.0)	128 (64.0)
Ciprofloxacin	200	47 (23.5)	104 (52.0)	49 (24.5)
Ceftazidime	200	84 (42.0)	101 (50.5)	15 (7.5)
Gentamicin	200	115 (57.5)	43 (21.5)	42 (21.0)
Nalidixic acid	200	22 (11.0)	65 (32.5)	113 (56.5)
Nitrofurantoin	200	81 (40.5)	75 (37.5)	44 (22.0)
Norfloxacin	200	67 (33.5)	56 (28.0)	77 (38.5)
Streptomycin	200	0 (0.0)	44 (22.0)	156 (78.0)
Trimethoprim	200	11 (5.5)	66 (33.0)	123 (61.5)
Tetracycline	200	2 (1.0)	20 (10.0)	178 (89.0)

p-ISSN: 2599-4875 e-ISSN: 2599-4980