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Prevalence and antibiotic resistance of *Salmonella spp*. and Escherichia coli isolated from poultry farms in the North Vietnam

Abstract

Salmonella spp. and *E. coli* isolated from 515 samples collected from poultry farms at the north Vietnam. The results showed that the presence of *E. coli* in the poultry farm was 59.4%. The highest positive rate was fecal samples 78.0%; following by sewages samples (77.8%), poultry house floors swap samples (68.0%), drinking water (17.8%), tools samples (16.7%) and none of 45 food samples were *E. coli* positive isolation. In this study, 37 samples (7.2%) were *Salmonella* positive isolation including 10.0% fecal samples; 9.3% poultry house floors swap and 6.7% seweages samples. The *E.coli* isolates were highly resistant to tetracycline (85.3%), streptomycin (83.3%), ampicillin (61.8%), trimethoprim (56.9%) and nalidixic acid (55.9%). They were low resistant to ceftazidime (6.9%) and nitrofurantoin (12.7%). The other antibiotics such as norfloxacin, ciprofloxacin and gentamicin were resisted by *E.coli* isolates range from 15.7% to 32.4%. In our results, the *Salmonella* isolates were commonly resistant to streptomycin (89.2%), tetracycline (83.8%) and ampicillin (59.5%). Three antibiotic such as ciprofloxacin, gentamicin and norfloxacin were resisted by the *Salmonella* isolates with the similar rates (21.6%). *Salmonella* isolates showed resistance to ceftazidime at the lowest rate (16.2%). Of the isolated trains, 65.7% of the *E. coli* and 62.2% of the *Salmonella* isolates showed multi-drug resistance. The data suggest that detection of resistance isolates from chicken, poultry environment, and humans need for one health consideration in the usage of antibiotics in the poultry industry.

Keywords: antibiotic resistance, *E. coli*, poultry farms, *Salmonella Spp*, vietnam

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Introduction

Antibiotics have been used widely in in farm animals, especially for poultry production to treat and prevent bacterial infections and as growth promoters in feeds.[1–3] However, it is now generally known that the widespread use of antibiotics is the main risk factor for an increase in the occurrence of bacterial resistant strains.[4,5] The emergence and dissemination of antibiotic-resistant bacterial strains like *Salmonella* and Escherichia coli from poultry products are reported to be high and are increasing worldwide.[3,6] Beside, antibiotic-resistant *Salmonella* and *E. coli* strains involved the human diseases are mostly spread from food and farm animals.[7–9] Therefore, it is necessary to know where the origin sources of antibiotic-resistant bacteria which were contaminated to the food production chains; and any knowledge about that might provide valuable information to reduce the sources of contamination with resistant bacteria during food processing and thus to minimize the risk for the consumer. Thus, given the health risks linked to the presence of *E. coli* and *Salmonella* in both poultry production and human health, as well as the antibiotic resistance issue posed by these germs in public health, regular monitoring of their resistance to antibiotics in poultry farming is essential.[1,10]

However, as far as we are aware, there have not been any reports about the occurrence of antibiotic-resistant bacteria in commercial poultry farms in Vietnam. The aim of this study is to detect the prevalence and antibiotic resistance rates of *Salmonella* and *E. coli* at poultry farms in the North Vietnam in order to provide the useful information for the food safety and public health in Vietnam.

Material and methods

For the samples collection, during three years, from January 2011 to December 2013, 515 samples collected from 45 flocks of 15 poultry farms located in three provinces (Bac Ninh, Hai Duong, Vinh Phuc) and two cities (Ha Noi, Hai Phong) at the north Vietnam including fecal samples (n=200), poultry house floors (n=150), drinking waters (n=45), poultry foods (n=45), sewages (n=45) and tools (n=30); Swab samples (poultry house floors, tools) were sampled by autoclaved cottons 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 and sewages were taken to 200 mL bottle and then 10 mL was mixed with 90 mL of BPW; approximately 10 gram of fecal samples and 250 gram of poultry food samples were taken to the sterilized plastic bags. All samples were kept on ice box during the transportation and characterized on the arrival day at the laboratory of Department of Veterinary hygiene, National of Institute veterinary research, Vietnam.

For the bacteria isolation, at the laboratory 1 gram of substance from fecal samples; 25 gram of food samples were homologized with BPW following the ratio 1:9; the bags of swap samples, drinking and seweages samples at the samples collection step also incubated at 370C within 18 – 24 hr for pre-enrichment. The next steps for *Salmonella* isolation were previously described.[11] For *E. coli* isolation, 0.1 mL of the 18 - 24h incubated pre-enrichment cultures was continuously incubated onto eosin methylene blue agar (EMB) at 37°C for 24 hr. Only one typical colony producing metallic sheen on EMB agar were isolated and streaked into the triple sugar iron agar (TSI) tube and incubated at 37°C for 24 hr. The colonies in TSI agar showed typical *E. coli* behaviors, 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 utilisation, indol production, methyl red and Voges-Proskauer reactions.

For the antibiotic susceptibility testing, in 2021, thirty seven isolated *Salmonella* and one hundered isolated *E.coli* strains were

randomly selected to characterize the antibiotic susceptibility at the Laboratory of the Faculty of Veterinary medicine, Vietnam national university of agriculture, Hanoi, Vietnam. The antibiotic susceptibility of isolates was determined according to the guidelines of the Clinical and Laboratory Standards Institute.[12] 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 (Am), 10 µg; ceftazidime (Cf), 30µg; ciprofloxacin (Ci), 5µg; gentamicin (Ge), 10µg; nalidixic acid (Na), 30 µg; norfloxacin (Nor), 10µg; streptomycin (St), 10µg; tetracycline (Te), 30µg; trimethoprim (Tm), 5 µg and nitrofurantoin (Ni), 300µg. The susceptible, intermediate or resistant interpretive categories were used according to CLSI guidelines.[13] Multi drug-resistant (MDR) strains were defined when it showed the resistance to at least one antibiotic in three or more different categories.[14] In our study, antibiotic classes included: Aminoglycosides (gentamicin, streptomycin); β - Lactams (ampicillin, ceftazidime); Pyrimidine (trimethoprim); Quinolones (ciprofloxacin, norfloxacin, nalidixic acid); Tetracyclines (tetracycline) and Nitrofuran (nitrofurantoin).

Data and statistical analysis, statistical comparison of the prevalence, antibiotic resistance rates between *Salmonella* and Escherichia coli from different sources was analyzed by the Chi-square test (Microsoft Excel 2016).

Results

The percentage of *E.coli* and *Salmonella* positive samples collected from poultry farms shown in the Table 1. Overall, the presence of *E. coli* in the poultry farm was quite high, 306/515 (59.4%) examined samples. The highest positive rate was fecal samples 156/200 (78.0); following by seweages samples 35/45 (77.8%), then poultry house floors swap samples 102/150 (68.0%); the positive rates of drinking warter and tools samples were 8/45 (17.8%) and 5/30 (16.7%), respectively; and none of 45 food samples were *E. coli* positive isolation. Thirty seven of 515 (7.2%) samples were *Salmonella* positive isolation. Among them, *Salmonella* was detected from 20/200 (10.0%) fecal samples; the prevalence of *Salmonella* in poultry house floors swap and seweages samples were 14/150 (9.3%) and 3/45 (6.7%), respectively; there were no *Salmonella* contamination with the tools, poultry food and drinking samples.

Antibiotic susceptibility of the *E.coli* isolates were shown in the Table 2 and Table 3. The *E.coli* isolates were highly resistant to tetracycline (85.3%), streptomycin (83.3%), ampicillin (61.8%), trimethoprim (56.9%) and nalidixic acid (55.9%). They were low resistant to ceftazidime (6.9%) and nitrofurantoin (12.7%). The other antibiotics such as norfloxacin, ciprofloxacin and gentamicin were resisted by *E.coli* isolates range from 15.7% to 32.4%. A total of 29 antibiotypes were obtained from the *E. coli* isolates, only six strains shown the sensitivity to all of ten antibiotics. And 67/102 (65.7%) of the *E. coli* strains showed multi drug-resistant (resisted at least three different antibiotic classes). More than half (52/102 – 51.0%) were resistant to at least five antibiotics. Fifty five (14.7%) strain was recorded against five antibiotics belong to five different classes; only one isolate was resistant to all compounds tested. The most commonly found resistance patterns are listed in Table 4. The resistance profile was the most prevalent (14.7%), which was resistant to Na – Tm – Am – St – Te; the patterns Ge – Na – Tm – Am – St – Te, was observed in ten (9.8%) of the *E coli* isolates.

Antibiotic susceptibility of the *Salmonella* isolates were presented in the Table 4 and Table 5. The *Salmonella* isolates were commonly resistant to streptomycin (89.2%), tetracycline (83.8%) and ampicillin (59.5%). Three antibiotic such as ciprofloxacin, gentamicin and norfloxacin were resisted by the *Salmonella* isolates with the similar rates (21.6%). *Salmonella* isolates showed resistance to ceftazidime at the lowest rate (16.2%). The antibiotic resistance rates of nalidixic acid and trimethoprim were 43.2% and 54.1%, respectively. There were thirty six *Salmonella* strains among the thirty seven isolates that showed resistant to at least one antibiotics. And twenty seven different resistance patterns were recorded. In particular, 23/37 (62.2%) of the *Salmonella* isolates showed the multi drug-resistance. Sixty multi drug-resistant *Salmonella* isolates predominantly resisted to 3 - 7 antibiotics belong to 3 -5 different antibiotic classes, the predominant MDR profile was Na-Tm-Am-St-Te (10.8%) and No-Ci-Na-Tm-Am-St-Te (8.1%). Interestingly, there were four *Salmonella* strains could resisted to 8 – 10 antibiotics belong to 6 different antibiotic classes.

Table 1 The prevalance of *Salmonella spp* and *Escherichia coli* isolated at poultry farms

No.	Samples	No. of examined samples	E. coli n (%)	Salmonella n (%)
1	Fecal samples	200	156 (78.0)	20 (10.0)
2	Poultry house floors	150	102 (68.0)	14 (9.3)
3	Tools	30	5 (16.7)	0 (0.0)
4	Poultry food	45	0 (0.0)	0 (0.0)
5	Drinking warter	45	8 (17.8)	0 (0.0)
6	Seweages	45	35 (77.8)	3 (6.7)
7	**Total**	**515**	**306 (59.4)**	**37 (7.2)**

Table 2 The antibiotic resistance of *Escherichia coli* isolated at the poultry farms (n = 102)

No.	Antibiotics	Results		
		Sensitivity n (%)	Intermediate n (%)	Resistance n (%)
1	Ampicillin	8 (7.8)	31 (30.4)	63 (61.8)
2	Ciprofloxacin	62 (60.8)	21 (20.6)	20 (19.6)
3	Ceftazidime	64 (62.7)	31 (30.4)	7 (6.9)
4	Gentamicin	51 (50.0)	18 (17.6)	33 (32.4)
5	Nalidixic acid	19 (18.6)	26 (25.5)	57 (55.9)
6	Nitrofurantoin	53 (52.0)	36 (35.3)	13 (12.7)
7	Norfloxacin	59 (57.8)	27 (26.5)	16 (15.7)
8	Streptomycin	0 (0.0)	17 (16.7)	85 (83.3)
9	Trimethoprim	11 (10.8)	33 (32.3)	58 (56.9)
10	Tetracycline	5 (4.9)	10 (9.8)	87 (85.3)

Citation: Huong CTT, Ngoc PT, Thai TH. Prevalence and antibiotic resistance of *Salmonella spp*. and Escherichia coli isolated from poultry farms in the North Vietnam. *J Dairy Vet Anim Res*. 2023;12(1):70–75. DOI: 10.15406/jdvar.2023.12.00326

Table 3 Antibiotic resistance pattents of *E. coli* trains isolated from the poultry farms

No.	Pattents	n(%)	No. of antibiotics resisitance	No. of antibiotics class
1	Na	4 (3.9)	1	1
2	St	2 (2.0)	1	1
3	Te	7 (6.9)	1	1
4	AmSt	1 (1.0)	2	2
5	StTe	15 (14.7)	2	2
6	GeStTe	1 (1.0)	3	2
7	TmAmSt	2 (2.0)	3	3
8	TmStTe	3 (2.9)	3	3
9	AmStTe	3 (2.9)	3	3
10	NiNaTe	1 (1.0)	3	3
11	NaAmStTe	4 (3.9)	4	4
12	TmAmStTe	1 (1.0)	4	4
13	NaTmAmStTe	15 (14.7)	5	5
14	GeTmAmStTe	3 (2.9)	5	4
15	GeNaTmAmStTe	10 (9.8)	6	5
16	NiGeTmAmStTe	1 (1.0)	6	5
17	CiNaTmAmStTe	2 (2.0)	6	5
18	NiGeNaTmAmStTe	2 (2.0)	7	6
19	CiGeNaTmAmStTe	2 (2.0)	7	5
20	NoGeNaTmAmStTe	2 (2.0)	7	5
21	NiCiGeNaTmAmStTe	1 (1.0)	8	6
22	NoCiGeNaTmAmStTe	3 (3.0)	8	5
23	CefNoGeNaTmAmStTe	2 (2.0)	8	5
24	NiNoGeNaTmAmStTe	1 (1.0)	8	6
25	CefNiNoCiNaTmAmStTe	2 (2.0)	9	6
26	CefNoCiGeNaTmAmStTe	1 (1.0)	9	6
27	NiNoCiGeNaTmAmStTe	3 (2.9)	9	6
28	CefNiNoCiNaTmAmStTe	1 (1.0)	9	6
29	CefNiNoCiGeNaTmAmStTe	1 (1.0)	10	7

Table 4 The antibiotic resistance of *Salmonella spp* isolated at the poultry farms (n = 37)

No.	Antibiotics	Results		
		Sensitivity n (%)	Intermediate n (%)	Resistance n (%)
1	Ampicillin	4 (10.8)	11 (29.7)	22 (59.5)
2	Ciprofloxacin	24 (64.9)	5 (13.5)	8 (21.6)
3	Ceftazidime	23 (62.2)	8 (21.6)	6 (16.2)
4	Gentamicin	25 (67.6)	4 (10.8)	8 (21.6)
5	Nalidixic acid	13 (35.1)	8 (21.6)	16 (43.2)
6	Nitrofurantoin	21 (56.8)	6 (16.2)	10 (27.0)
7	Norfloxacin	24 (64.9)	5 (13.5)	8 (21.6)
8	Streptomycin	0 (0.0)	4 (10.8)	33 (89.2)
9	Trimethoprim	12 (32.4)	5 (12.5)	20 (54.1)
10	Tetracycline	2 (5.4)	4 (10.8)	31 (83.8)

Table 5 Antibiotic resistance pattents of *Salmonella* strains isolated from the poultry farms

No.	Pattents	n (%)	No. of antibiotics resisitance	No. of antibiotics class
1	Na	1 (2.7)	1	1
2	St	2 (5.4)	1	1
3	Te	2 (5.4)	1	1
4	TmSt	2 (5.4)	2	2
5	StTe	6 (16.2)	2	2
6	AmStTe	3 (8.1)	3	3
7	NiAmStTe	1 (2.7)	4	4
8	NaTmAmStTe	4 (10.8)	5	5
9	NiNaTmStTe	1 (2.7)	5	5
10	CefNiAmStTe	1 (2.7)	5	4
11	CefGeTmAmStTe	1 (2.7)	6	4
12	NiGeTmAmStTe	2 (5.4)	6	5
13	CefGeNaTmAmStTe	1 (2.7)	7	5
14	NoCiNaTmAmStTe	3 (8.1)	7	5
15	CefNiGeTmAmStTe	1 (2.7)	8	5
16	CefNoCiNaTmAmStTe	1 (2.7)	8	5
17	NiNoCiNaTmAmStTe	1 (2.7)	8	6
18	NiNoCiGeNaTmAmStTe	2 (5.4)	9	6
19	CefNiNoCiGeNaTmAmStTe	1 (2.7)	10	6

Citation: Huong CTT, Ngoc PT, Thai TH. Prevalence and antibiotic resistance of *Salmonella spp*. and Escherichia coli isolated from poultry farms in the North Vietnam. *J Dairy Vet Anim Res*. 2023;12(1):70–75. DOI: 10.15406/jdvar.2023.12.00326

Discussion

Salmonella contamination of poultry and derivative products occurs at different levels. Thirty seven (7.2%) of the 515 poultry farms samples were contaminated with *Salmonella*. The isolation rate varied according to the origin of the sample, 10.0% for fecal samples, 9.3% for poultry house floor swaps and 6.7% for the seweage samples. The other report in Vietnam[15] shown that, *Salmonella* was present in the poultry farms samples such as dinking warter, bedding, feed, chicken feces and environmental samples ranged from 0.7% to 7.7%. Around the world, the *Salmonella spp*. prevalence varied considerably among regions and countries such as in several European countries. The reported prevalence was in France (3.4%), Italy (9.2%), Germany (2.7%), Spain (1.02%), Poland (1.57%) and Sweden (nearly 0%).[16–18] The isolation rate varied from 2.8% to 15.9% according to the origin of the sample at the Afican countries such as Algeria, Ethiopia and Nigeria.[19–21] The prevalence of *Salmonella* in broiler farms at Colombia[22] was higher than that in our research (26.7%). However, *Salmonella* was only isolated in 6.7% of the poultry litter samples in the southern United States.[7] The prevalence of non-typhoidal *Salmonella* can be found in environmental reservoirs, infections from which are challenging to control.10 In chicken farms, *Salmonella* can be transmitted through feces, vehicles, workers, clothing, footwear, equipment, water, food, garbage, animals, and other factors.[23]

In this study we examined antibiotic resistance in commensal *E. coli* isolates from chicken farms in some provices and cities in the north of Vietnam. The high level of *E. coli* resistance in chicken farms is a public health concern, this may also be an indicator of emerging resistance in other gut microflora within the chicken population. The isolates were highly resistant to tetracycline (85.3%), streptomycin (83.3%), ampicillin (61.8%), trimethoprim (56.9%) and nalidixic acid (55.9%). High resistance of *E.coli* isolated from chicken farms to broad-spectrum antibiotics have been reported in Vietnam.[15] The authors reported that *E. coli* were highly resistant to tetracycline (93.4%), and ampicillin (86.0%), and Trimethoprim/sulfamethoxazole (69.7%). The significant proportion of resistance against the above antibiotics was also reported from Ghana, Nigeria and China.[24–26] The observed high prevalence of antibiotic resistance reflects the common use of antibiotic products for therapeutic and prophylactic purposes, as found in our survey on antibiotic drug usage.[18] In contrast, in this study, isolated *E.coli* strains showed relatively lower grade of resistance against third generation cephalosporins (ceftazidime – 6.9%), quinolones group (norfloxacin – 15.7% and ciprofloxacin – 19.6%). This finding is supported by other studies showing lower resistance rates against the above antibiotics.[27–29] However, data from seven European countries suggest a higher prevalence of ciprofloxacin resistance (57.6%), while data from five European countries indicate a higher prevalence of ceftazidime resistance (11.1%) in bacteria isolated from chickens in these countries.[30,31] Although such comparisons should be interpreted with caution because of differences in sampling methods as well as differences in the breakpoints used for interpreting susceptibility test results between studies from different regions.

Antibiotic resistance may arise due to the indiscriminate use of antimicrobials and their use as growth promoters and chemotherapeutic agents to control diseases at farms.[18] Most *Salmonella* isolates in this study showed relative resistance to streptomycin (89.2%), tetracycline (83.8%) which have been used to treat salmonellosis since many years in Vietnam. The use of antibiotics over long periods thus favors the selection of resistant bacterial strains. The high percentage of *Salmonella* isolates resisted to the antibiotic above in current research is in line with the previous reports in Bangladesh and Vietnam.[32,33] In curent research, the *Salmonella* isolates resisted to ampicillin at high level rate (59.5%). Our results were in line with reports from Myanmar, 47.1%; Malaysia, 72.7% and Singapore, 78.8%;[34–36] however, *Salmonella spp*. resistance to ampicillin was observed at lower rates in Japan, 17.9%; South Korea, 5.6% and Iran, 11.7%.[37–39] Quinolone resistance is a current worldwide problem in human and veterinary medicine. The *Salmonella* isolates resisted to ciprofloxacin, norfloxacin and nalidixic acid by range from 21.6% to 43.2%. In comparison to the other results, higher rates of quinolones resistance (ciprofloxacin and nalidixic acid) have been reported in *Salmonella* isolated from poultry in the EU (64.7% and 61.5%), China (25.7% and 46.7%) and Brazil (86.5% and 89%).[30,40,41] However, *Salmonella* isolated from broilers have shown a low resistance frequency to quinolones in Canada (3% and <1%) and in the USA (0% and <1%), which can be attributed to the restricted use of fluoroquinolones in poultry.[2,42,43]

Emergence of MDR bacteria, especially Enterobacteriaceae, has increased in recent years. In this study, 65.7% of the *E. coli* and and 62.2% of the *Salmonella* isolates showed MDR. The similar proportions of MDR were also reported for Salmonela isolates from Bangladesh, Nigeria and Malaysia;[44,45,32] and for *E. coli* isolates from Vietnam, Malaysia and Ghana.[45,24,46] The occurrence of MDR may be linked with indiscriminate use of antimicrobial agents. MDR makes the difficult treatment of infections caused by pathogenic bacteria in both poultry and humans. Thus, the use of antibiotics at poultry farms and households should be controlled to prevent the creation of MDR pathogenic strains.[47-51]

Conclusion

In this study, the prevalence of *E. coli* and *Salmonella spp*. detected in chicken farms in the north Vietnam was 59.4% and 7.2%, respectively. The isolates showed high resistance rates to tetracycline, ampicillin, strepmycin and trimethoprim; and its were fairly susceptible to ceftazidime, norfloxacin, ciprofloxacin. This varying degree of resistance shown by *E. coli* and *Salmonell spp*. supports the assertion that the poultry environment is a potential reservoir for antibiotic-resistant trains that can spread from the animals to the human population using poultry liter for farming purposes. In Vietnam, like in other developing countries, the indiscriminate and widespread use of antimicrobials in veterinary practice and the easy access to antimicrobials by farmers who can purchase them without any prescription should be addressed. These findings provide evidence for the emergence for the antimicrobial resistance of *Salmonella spp*. and *E. coli* in poultry farms in Vietnam. Future studies should focus on detection of resistance isolates from chicken, poultry environment, and humans to demonstrate the need for one health consideration in the usage of antibiotics in the poultry industry.

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Conflicts of Interest

Author declares there is no conflict of interest in publishing the article.

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