Antimicrobial resistance of *Salmonella* serovars isolated from meat shops at north Vietnam

（ベトナム北部の食肉店から分離されたサルモネラ菌の抗菌耐性に関する研究）

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Contents

1. Preface

1. 1. *Salmonella* and food-borne disease

The genus *Salmonella* belongs to the family *Enterobacteriaceae* whose members are Gram-negative, nonspore-forming, facultatively anaerobic, glucose fermenting, nitrate reducing, oxidase-negative, and straight rods of about 0.7-1.5 x 2.0-5.0 μm. Most *Salmonella* are motile via peritrichous flagellae except *S.* Gallinarum and *S.* Pullorum. The genus *Salmonella*, first known as *Salmonella choleraesuis*, was initially discovered in 1886 by Theobald Smith and Daniel Elmer Salmon. The discovery of the genus originated from the work on swine fever (hog cholera) by Theobald Smith and he named the genus after his supervisor at the U.S. Department of Agriculture (USDA), Daniel E. Salmon (Grimont et al., 2000).

Currently, there are over 2,500 identified *Salmonella* serotypes. Most serotypes share a very high degree of genetic similarity, and because of this similarity, the Salmonellae are now divided into just 2 species, *Salmonella enterica* and *Salmonella bongori.* Over 99% of the serotypes are grouped into the species *S. enterica,* which contains all of the major serovars that are pathogenic to humans (Popoff, 2001; Bopp et al., 2003; Yan et al., 2003). The species, *Salmonella enterica* is divided into six subspecies – *Salmonella enterica* subsp. e*nterica* (I), *Salmonella enterica* subsp. *salamae* (II), *Salmonella enterica* subsp. *arizonae* (IIIa), *Salmonella enterica* subsp. *diarizonae* (IIIb), *Salmonella enterica* subsp. *houtenae* (IV), and *Salmonella enterica* subsp. *indica* (VI). Serovars of *Salmonella enterica* subsp. *enterica* (I), are primarily named by geographical origin such as *S.* Amsterdam, *S.* Panama, and *S.* Derby whereas the serovars of the remaining five subspecies all are named by antigenic fomular (Grimont et al., 2000; Grimont et al., 2007).

Although all *Salmonella* are considered potentially pathogenic, the majority of human cases of non-typhoidal salmonellosis are caused by only a few serovars, such as *S.*

Enteriditis, *S.* Typhimurium, *S.* Anatum, *S.* Derby, *S.* Hadar, *S.* Rissen, *S.* Braenderup, *S.* Virchow, *S.* Javiana, *S.* Weltevreden, *S.* Infantis, *S.* Newport, *S.* Heidelberg etc… (Bangtrakulnonth et al., 2004; Galanis et al., 2006; Poppe et al., 2006; Hendriksen et al., 2011). *S.* Enteriditis and *S.* Typhimurium are the two most important serotypes for human salmonellosis (Galanis et al., 2006; Lynch et al., 2006; Hughes et al., 2007). These two particular strains with epidemic potential will continue to be the dominant serotypes worldwide in the foreseeable future (World Health Organization, 2005).

Salmonella infections are one of the most prevalent food-borne infections worldwide. Infections via animal origin foods especially poultry, meat, egg, and raw milk are the sources of the illness that *Salmonella* cause so called "salmonellosis". The patient has the symptoms stomachache, fever, diarrhoea, nausea and vomiting whereas life-threatening for the infants, elderly and immunocompromised patients. It is estimated that, in each year at the United States, about 1.4 million persons are infected with non-typhoidal *Salmonella*, which results in 15,000 hospitalisations and 580 deaths (World Health Organization, 2005). The worldwide incidence of non-typhoidal salmonellosis is estimated that about 1.3 billion cases and 3 million deaths annually (Tassios et al., 1997). In many regions such as South East Asia, there is an absence of official *Salmonella* surveillance data, but it is estimated that up to 22.8 million cases occur annually with 37,600 deaths (Majowicz et al., 2010). In recent years, the Vietnamese government has shown considerable attention to reducing the risks to food. With the country's population of more than 83 million and about three quarters of the population living in rural areas, the country will continue to have lots of challenges to face with foodborne diseases. However, the true incidence of foodborne disease is not known. Annual burden of food-borne disease in Vietnam is estimated at 128 million episodes of diarrhoea per year, of which 27 million cases had medical health care and 3.5 million had hospitalizations (McGregor-Skinner,

2004). The lack of skilled manpower in terms of food inspection, analytical capacity and surveillance, and the lack of appropriately equipped labs, limit knowledge about food safety of consumer etc., combined with rapid population and economic growth, urbanisation and globalisation of food trade, could lead to serious threats of foodborne disease for the Vietnamese citizens and overseas consumers (World Health Organization, 2005). The food administration, with assistance from World Health Organization (WHO), is developing its capabilities and programmes to achieve food safety goals.

1. 2. The use of antimicrobial in animal husbandry

Antimicrobials agents are essential for human health and animal and welfare because they can be used against infections caused by bacteria. Therefore, the increasing antimicrobials resistance seen today is a global public health concern. Antimicrobial misuse and excessive antimicrobial use in human and veterinary medicine are important reasons for the increasing and spread of antimicrobial resistance among the bacteria (Pappaioanou, 2004). The prevalence of antimicrobial resistance among food-borne pathogens has increased during recent decades (Aarestrup et al., 2008). In addition, the emergence of antimicrobial-resistant bacteria transmitted from an animal reservoir to humans in relation to the use of antimicrobial in food animals has been demonstrated (White et al., 2002). They underline the role of antimicrobial usage in food, prevention and treatments for animals in the selection of bacterial resistance and the transport of these resistances via the food chain to humans.

In food animal production, antimicrobials are used for disease treatment, disease prevention and growth promotion. The permitted uses vary among countries and regions; for example antimicrobials are no longer be used as animal growth promoters in European Union but these practice is permitted in many other countries (Gyles, 2008). Furthermore, the amount of antimicrobial usage per animal differs between countries as it also depends on

the effectiveness of regulation enforcement, and the knowledge of the community about antimicrobial use and resistance. Besides, the incidence of resistance to antimicrobials of bacteria originating from food animals or retail meat is high in developing countries (Fashae et al., 2010; Van et al., 2007; Yang et al., 2010), possibly as the result of the inappropriate or uncontrolled use of antimicrobial in farming practices.

In Vietnam, more than 3,000 pharmaceutical products are used in animals, including antimicrobials, vitamins and anti-parasites. Antimicrobials (about 70% of all products) were the most common registered by 70 veterinary medicine companies (Ministry of Agriculture and Rural Development, Vietnam, 2011). The pharmaceutical products were sold commonly by the veterinary medicine drug-stores in 63 provinces and cities, where antimicrobials are available "over the counter", the control of antimicrobial use is insufficient. The understanding in the community of prudent use of antimicrobials is limited (Larsson et al., 2000; Larsson, 2003; Van Nhiem et al., 2006). Antimicrobials were widely used in animal husbandry for therapy and prevention of bacterial infections and breeders normally use antimicrobials to treat sick animal with higher dose and using their own experience without veterinary prescription, supervision and laboratory diagnosis (Van Nhiem et al., 2006). In addition, almost of the antimicrobials used for animals are also used to treat human infection diseases in Vietnam such as neomycin, streptomycin, penicillin, norfloxacin, trimethoprim, erythromycin… (Vo et al., 2010; Dang et al, 2011). Several lines of evidence indicate that antimicrobial resistance in animal husbandry results from the use of antimicrobials in food animals (Rosenfren et al., 2007; Harada et al., 2008; Thakur et al., 2007; Miranda et al., 2008). It may suggest that the high prevalence of antimicrobial resistance in the bacteria isolates from both human and veterinary side in Vietnam recently (Van et al., 2007; Ogasawara et al., 2008; Vo et al., 2010) may link to the widespread of antimicrobial use in animal husbandry.

1. 3. Antimicrobial resistance and antimicrobial resistance mechanism in *Salmonella*

Although most *Salmonella* infections are self-limited, causing acute gastrointestinal illness in humans, antimicrobial agents are commonly prescribed to those seeking medical attention. Severe infections that spread to the bloodstream, meningeal linings of the brain or other deep tissue can also occur. The selection of effective antimicrobials is critical for the treatment of invasive infections, but has become more difficult as antimicrobial-resistance has increased (Angulo et al., 2000). The use of antimicrobials not only selects for antimicrobial-resistant bacteria, but may also increase the likelihood of disease transmission. Antimicrobial resistance in non-typhoid *Salmonella* serotypes has been a global problem. Surveillance data demonstrated an obvious increase in overall antimicrobial resistance among salmonellae from 20%–30% in the early 1990s to as high as 70% in some countries at the turn of the century (Su et al., 2004). The resistance rate, however, varies with different serotypes and different antimicrobials. Serovar Enteritidis, which is one of the most prevalent *Salmonella* serovars, is relatively more susceptible to antimicrobial agents than other serovars. A much higher rate of resistance was found in serovar Typhimurium, which is another globally prevalent serovar (Su et al., 2004).

During recent years, antimicrobial multi-resistance has been documented in various *Salmonella* serovars and a large number of antimicrobial resistance genes have been identified by molecular approaches, including hybridization experiments and PCR analysis (Micheal et al., 2006; Strahilevitz et al., 2009; Rodríguez-Martínez et al., 2011).

Antimicrobial resistance arises in several ways, including acquisition of resistance genes via horizontal gene transfer and selection of resistant variants in the population. In the case of *Salmonella*, the situation is more complicated, because the use of antimicrobials for therapeutic or preventive purposes in veterinary medicine and as growth promoters in animal feed may promote the emergence of resistance, thereby presenting a potential risk to public

health from zoonotic infections (McEwen and Fedorka-Cray, 2002). Although the route of transmission of antimicrobial-resistant *Salmonella* is complex, evidence obtained in many epidemiological and laboratory studies suggest that the primary source of antimicrobial-resistant *Salmonella* infection is foods of animal origin (Swartz, 2002). Recent surveys with molecular techniques provide firm evidence indicating that the use of antimicrobial agents in food animals contributes to the development of antimicrobial-resistant salmonellae that cause infections in humans (Dunne et al., 2000; Fey et al., 2000; McEwen and Fedorka-Cray, 2002; Swartz, 2002).

Widespread drug-resistant non-typhoid *Salmonella* and the associated complications in the treatment of infection have constituted a serious threat to the public health. Treatment with conventional antimicrobials, such as ampicillin, chloramphenicol, and sulfamethoxazole-trimethoprim, is now difficult, and the increasing resistance to newer antimicrobial agents, such as fluoroquinolones and extended-spectrum cephalosporins, further aggravates the problem (Su et al., 2004; Van et al., 2012). One of the means of overcoming the resistance problem in *Salmonella* is to discontinue the overuse and misuse of antimicrobial agents in food animals (Chiu et al., 2002; McEwen and Fedorka-Cray, 2002; Swartz, 2002; Aarestrup et al., 2001; Evans et al., 2003). Furthermore, because the resistance displayed by *Salmonella* serotypes reflects the environment, in which the organism thrives, immediate action, including rigorous restriction of the use of extended-spectrum cephalosporins and fluoroquinolones in food animals and humans and reinforcement of infection-control measures in clinical settings, should also be taken. Continued surveillance for resistance and the inclusion of appropriate screening tests for extended-spectrum cephalosporinases in routine susceptibility testing for *Salmonella* are necessary.

This study was attempted to obtain more detailed information on the *Salmonella* strains isolated from meat shops at retail markets at the north Vietnam: technical-based for

Salmonella detection, antimicrobial resistance testing and PCR- based methods were used. Furthermore, detection of antimicrobial resistance genes and mutation by using polymerase chain reaction, sequencing methods were also performed.

Objectives

The overall aim of this study was to determine the *Salmonella* contamination at meat shops at the north Vietnam and their ability of antimicrobial resistance and antimicrobial resistance mechanism.

Specifically, the objectives were:

i) To determine the prevalence of *Salmonella* contamination at the meat shops.

ii) To determine the prevalence of antimicrobial resistance and antimicrobial resistance profiles.

iii) To determine the resistance genes and mutation encoding for antimicrobial resistance by using polymerase chain reaction (PCR) and sequencing methods.

iv) To determine the plasmid encoding for quinolone-resistant strains.

2. Chapter I: Antimicrobial resistance of *Salmonella* serovars isolated from pork and chicken meat at retail markets in the north Vietnam

Abstract

During 2007 - 2009, a total of 586 retail meat samples (318 pork and 268 chicken meats) were collected at retail markets from three provinces (Bac Ninh, Ha Noi and Ha Tay) in north Vietnam. Approximately 39.6% (n=126) of pork and 42.9% (n=115) of chicken samples were *Salmonella*-positive, and 14 *Salmonella* serovars were identified. Anatum (15.8%) was the most common serovar, followed by Infantis (13.3%), Emek (10.4%), Derby and Rissen (9.5%), Typhimurium (9.1%), Reading (7.5%) and London (6.2%). The isolation frequency of serovars Enteritidis, Albany, Hadar, Weltevreden, Newport and Blockey ranged from 1.2%-5.8%. Resistance to at least one antimicrobial was detected in 78.4% (n=189) of isolates. The highest resistance was to tetracycline (58.5%), followed by sulphonamides (58.1%), streptomycin (47.3%), ampicillin (39.8%), chloramphenicol (37.3%), trimethoprim (34.0%) and nalidixic acid (27.8%). None of *Salmonella* isolates were resistant to ceftazidime. The chicken isolates had higher resistance to antimicrobials than the pork isolates ($P<0.05$). Our research showed that 134 *Salmonella* isolates belong to the 14 serovars were multidrug resistance (MDR) and 47 MDR patterns were found. Fourteen [bla_{TEM}, bla_{OXA-1}, bla_{PSE-1}, *aadA1*, *sul1*, *tetA*, *tetB*, *tetG*, *cmlA1*, *floR*, *dfrA1*, *dfrA12*, *aac(3)-IV*, and *aphA1-1AB*] of 17 resistance genes were detected from the isolates demonstrating resistance. Plasmid-mediated quinolone resistance, such as *qnrA*, *qnrB*, *qnrS*, *qepA*, and *acc(6')-1b-cr* were not detected in 23 quinolone-resistant isolates. The substitution *TCC* to *TTC* at codon 83 of *gyrA* was found in the 18 quinolone-resistant isolates. The data revealed that resistant *Salmonella* strains were widely distributed in northern Vietnam via the food chain and that they might contain multiple genes specifying identical resistance phenotypes. Thus, further studies are necessary to clarify the mechanisms of antibiotic resistance in *Salmonella* strains and their spread in the livestock market.

2. 1. Introduction

Foodborne diseases caused by non-typhoid *Salmonella* are important global public health concern. Most human *Salmonella* outbreaks were associated with the consumption of contaminated animal-derived products (World Health Organization, 2005). The global human health impact of non-typhoidal *Salmonella* is high, with an estimated 93.8 million illnesses, of which an estimated 80.3 million are foodborne and associated with 155,000 deaths each year (Majowicz et al., 2012). Pork, beef and chicken meat have been recognized as significant sources of human salmonellosis. Although more than 2500 serovars of *Salmonella enterica* have been identified; however, most human *Salmonella* infections are caused by a limited number of serovars, which may vary from country to country and over time (Hendriksen et al., 2011).

The emergence of antimicrobial-resistant *Salmonella* has become a major public health concern. The use of antimicrobials in any venue, including disease treatment and growth promotion in domestic livestock, can potentially lead to widespread dissemination of antimicrobial-resistant bacteria. In recent years, studies on *Salmonella* isolates from foodstuffs and animals in Vietnam and other countries has shown that multidrug resistance of *Salmonella* is increasing (Van et al., 2007; Vo et al., 2010; Yang et al., 2010; Wannaprasat et al., 2011).

The increased use of fluoroquinolones has led to increasing resistance to these antimicrobials, with rates of resistance that vary by both organism and geographic region (Jacoby et al., 2005; Gay et al., 2006). The main mechanism of quinolones resistance was believed to arise from chromosomal mutations in genes encoding target enzymes: DNA gyrase (*gyrA* and *gyrB*) and/or DNA topoisomerase IV (*parC* and *parE*); or affecting drug accumulation (Jacoby et al., 2005; Gay et al., 2006; Strahilevitz et al., 2009). Besides mutations in genes chromosomal, plasmid mediated quinolone resistance (PMQR) has also been reported, including a *qnr*-mediated inhibition of quinolone binding to DNA, a *qepA* encoded efflux pump, and the *aac(6')-1b-cr* mediated

inactivation of fluoroquinolones by an acetyltransferase (Robicsek et al., 2006; Strahilevitz et al., 2009; Rodríguez-Martínez et al., 2011).

The aim of this study was to determine the *Salmonella* contamination rates, and to characterise antimicrobial resistance, antimicrobial resistance mechanisms in *Salmonella* serovars isolated from fresh meats at the retail markets in the north Vietnam.

2. 2. Materials and methods

2. 2. 1. Sampling methods, Salmonella isolation and serotyping

Sampling method: A total of 586 raw meat samples (318 pork and 268 chicken meats) was randomly collected from 586 meat shops at 83 retail markets in three provinces (Bac Ninh, Ha Noi and Ha Tay) of the north Vietnam from July 2007 to June 2009. Only one sample of meat was collected from one meat shops. Samples (>100 g) were placed to sterile plastic sampling bags and chilled in an ice box during transport to the laboratory at Hanoi University of Agriculture. All samples were analysed on the day of arrival.

Salmonella isolation: Salmonella strains were isolated according to the Standard ISO-6579 method (International Organization for Standardization, 1993) with some modifications. For pre-enrichment of *Salmonella*, 25 g of each sample was homogenized in sterile bags with 225 ml of buffered peptone water (BPW) and incubated at 37°C for 18 - 24 h. Next, 0.1 ml of the pre-enriched culture in BPW was added to 10 ml of Rappaport - Vassiliadis soya (RVS) broth followed by further incubation at 41.5°C for 24 h. A loopful of culture broth was then sampled from the selective enrichment RVS broth, streaked onto xylose-lysine-tergitol 4 agar (Merck), and incubated at 37°C for 24 h. Presumptive black colonies were selected from each plate and cultured on nutrient agar slants. The isolates were confirmed to be *Salmonella* by confirmatory biochemical tests (fermentation of glucose, lactose and sucrose; hydrogen sulphide production test, citrate test, lysine decarboxylation, and methyl red and indole tests).

Salmonella serotyping: 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) used by the Department of veterinary hygiene, National of institute veterinary research, Vietnam.

2. 2. 2. Antimicrobial susceptibility testing

The antimicrobial susceptibility of isolates was determined according to the guidelines of the Clinical and Laboratory Standards Institute, 2006a. Agar diffusion assays were performed on Muller - Hinton agar with disks containing 14 different antimicrobial agents (Oxoid, UK). The antimicrobials tested were as follows: ampicillin, 10 μg; amoxicillin/clavulanic acid, 20/10 μg; ceftazidime, 30 μg; chloramphenicol, 30 μg; ciprofloxacin, 5 μg; gentamicin, 10 μg; kanamycin, 30 μg; nalidixic acid, 30 μg; neomycin, 10 μg; norfloxacin, 10 μg; streptomycin, 10 μg; tetracycline, 30 μg; trimethoprim, 5 μg and sulphonamides 300 μg. The interpretive categories susceptible, intermediate or resistance were used according to the Clinical and Laboratory Standards Institute guidelines, 2010; except for neomycin where the zone criteria of ≤ 12 (resistant), 13-16 (intermediate) and ≥ 17 (susceptible) were determined (Haley and Prescott, 2002). *Escherichia coli* ATCC 25922 was used as the quality control organisms. An isolate was defined as 'resistance' after confirmation of resistance to at least one antimicrobial agent, while 'multiple resistance' was defined as resistance to three or more antimicrobial agents.

2. 2.3. Detection of resistance genes and mutations at gyrA

For resistance genes and mutation detection: Only the antimicrobial-resistant *Salmonella* strains collected from July 2008 to June 2009 at 283 meat shops in 45 retail markets at three provinces (listed in the Appendix 1) were further characterised their antimicrobial resistance mechanisms.

DNA preparation: DNA templates used for PCR were prepared by boiling bacterial cultures (Shahada et al., 2006). Cloned isolates were cultured overnight on brain–heart

infusion agar and several colonies were collected and transferred into 1mL of deionised distilled water. The microtubes were vortexed for 10s, the turbid bacterial suspension was centrifuged at 12,000 rpm for 2 min and the supernatant was discarded leaving behind a pellet. Thereafter, 100 µL of Insta Gene Matrix on a stirrer was added to each microtube, vortexed for 10s and incubated at 56^0C for 15 min in a water-bath. The suspension was vortexed for 10s, boiled at 100^0C for 8 min and centrifuged at 12,000 rpm for 2 min. Finally, 70 µL of the supernatant was carefully withdrawn and mixed thoroughly with 280 µL of sterile deionised distilled water and stored at -20^0C until required.

Detection of resistance genes: The following genes implicated with antimicrobial resistance were detected by PCR amplification: bla_{PSE-1}, bla_{OXA-1}, and bla_{TEM} encoding β-lactam resistance; *aadA1*, *aadA2*, *aac(3)-IV*, *aphA-1AB* and *Kn* encoding aminoglycoside resistance; *catA1*, *cmlA1* and *floR* encoding chloramphenicol resistance; *sul1* encoding sulphonamides resistance; *tetA*, *tetB*, *tetG* encoding tetracycline resistance; *dfrA1*, *dfrA12* encoding trimethoprim resistance. In the case of the isolates showing resistance to quinolone based antimicrobial compounds, an MIC for nalidixic acid was examined by broth dilute method according to the Clinical and Laboratory Standards Institute guidelines, 2006b. The PMQRs such as *qnrA*, *qnrB*, *qnrS, qepA, aac(6')-1b-cr* and QRDRs of *gyrA* was presented for quinolone resistance. PCR amplification reactions were performed in a 25µl volume of reaction mixture containing 12.5 µl of GoTaq®Green Master Mix, 2x (Promega, USA), 1 µl (10 ng/µl) of primers, 4 µl of DNA template and nuclease-free water. The primer sets and the assay conditions used for amplification were shown in the Appendix 2. The PCR products were analyzed by electrophoresis with 1.5% agarose in 1×Tris-boric acid-TBE buffer. The gels were stained with 1 µg/ml ethidium bromide, and visualized bands were photographed using a Polaroid camera on an ultraviolet light transilluminator. A molecular weight standard ladder was included on each gel (Toyobo, Japan).

Sequencing of gyrA: PCR amplifications of the quinolone resistance determining regions (QRDRs) to detect mutations at condon Gly 81, Ser 83 and Asp 87 in *gyrA* were carried out using the primers in previous report (Fàbrega et al., 2009). Purified PCR products were sequenced and compared with the wild-type sequences (GenBank accession number X78977.1).

2. 2. 4. Data and statistical analysis

Statistical comparison of antimicrobial resistance rates among serovars from different sources was analysed by the Chi-square test (Microsoft Excel 2003).

2. 3. Results

Salmonella isolation: Approximately 39.6% (n = 126) of pork and 42.9% (n = 115) of chicken meat samples were *Salmonella*-positive, and 14 *Salmonella* serovars were identified (Table 1). Anatum (15.8%) was the most common serovar, followed by Infantis (13.3%), Emek (10.4%), Derby and Rissen (9.5%), Typhimurium (9.1%), Reading (7.5%) and London (6.2%). The prevalence of serovars Enteritidis, Albany, Hadar, Weltevreden, Newport and Blockey ranged from 1.2% –5.8%.

Antimicrobial resistance: Antimicrobial resistance of the *Salmonella* isolates to 14 antimicrobials was shown in Table 2. Resistance to at least one antimicrobial agent was found in 78.4% (n = 189) of isolates (Table 3). None of the isolates were resistant to ceftazidime. The most resistance were tetracycline (58.5%), followed by sulphonamides (58.1%), streptomycin (47.3%), ampicillin (39.8%), chloramphenicol (37.3%), trimethoprim (34.0%) and nalidixic acid (27.8%). The *Salmonella* isolates had low resistance to amoxicillin-clavulanic acid (2.1%), norfloxacin (1.2%) and ciprofloxacin (5%).

Among *Salmonella* serovars isolates, the resistance to tetracycline, sulphonamides, streptomycin, chloramphenicol and ampicillin was frequently observed. Typhimurium, Infantis and Anatum had higher resistance rates to ampicillin than Emek, Derby, Rissen and London ($P < 0.05$). Infantis, Typhimurium and Emek had higher resistance rates to

sulphonamides than Anatum, Reading and London ($P < 0.05$). Overall, Typhimurium and Infantis had the highest antimicrobial resistance rates. Because of the limited number, antimicrobial resistance comparisons of Weltevreden, Hadar, Albany and Enteritidis to other serovars were not conducted.

Tables 3 and 4 showed multidrug resistance (MDR) of *Salmonella* serovars. In total, 134 MDR *Salmonella* isolates belonging to 14 serovars and 47 MDR patterns were identified. Resistance to 7 - 9 antimicrobials was detected in 36 isolates (14.9%) while 20 isolates (8.3%) were resistant to more than nine antimicrobials. MDR was widespread among different *Salmonella* serovars isolated from retail meat samples and frequently found in the serovars Typhimurium, Infantis, Anatum, Rissen and Emek. MDR to more than six antimicrobials was higher in chicken *Salmonella* isolates (n = 35) than that in pork *Salmonella* isolates (n = 21) ($P < 0.05$).

Detection of resistance genes: Fourteen (*bla*$_{TEM}$, *bla*$_{OXA-1}$, *bla*$_{PSE-1}$, *aadA1*, *sul1, tetA*, *tetB, tetG, cmlA1, floR, dfrA1, dfrA12, aac(3)-IV* and *aphA1-1AB*) of 17 resistance genes were detected from the resistant isolates collected from July 2008 to June 2009 (Table 5). Among the 39 ampicillin-resistant isolates, the *bla*$_{TEM}$ and *bla*$_{OXA-1}$ genes were detected in 36 (92.3%) and 9 (23.1%) isolates, respectively. Of the 64 tetracycline-resistant isolates, 47 (73.4%) were positive for *tetA*, 20 (31.3%) for *tetG* and 1 (1.6%) for *tetB;* of which, 16 (25%) carried both the *tetA* and *tetG* genes. The *sul1* gene was detected in 47 (75.8%) out of the 62 sulphonamides-resistant isolates. None of *aadA2* gene was found in the 49 streptomycin-resistant isolates; instead, *aadA1* gene was found in 41 (83.7%) of the resistant isolates. Among the 31 kanamycin-resistant isolates, 100% were positive for the *aphA1-Iab* gene. Of the 18 gentamicin-resistant isolates, 17 (94.4%) carried the *aac(3)-IV* gene. Among the 42 chloramphenicol-resistant isolates, none of the isolates contained the *catA1* gene; *floR* and *cmlA1* genes were detected in 29 (69%) and 24 (57.1%) isolates, respectively. The antimicrobial resistance genes were widely distributed in the *Salmonella* serovars isolates.

Detection of the PMQR genes and substitutions in gyrA of the quinolone-resistant isolates: PMQR genes such as *qnrA*, *qnrB*, *qnrS*, *qepA* and *acc(6')-ib-cr* were not detected from 23 quinolone-resistant isolates (Table 6). Sequence analysis of the *gyrA* revealed that 18 of 23 isolates had substitution at codon *Ser83* (*TCC* to *TTC*) with the MIC levels of nalidixic acid ≥128 µl/ml. In addition, all of the quinolone-resistant isolates in this study showed multidrug resistance and carried multiple resistance genes.

2. 4. Discussion

The *Salmonella* contamination rates were approximately 39.6% in pork and 42.9% in chicken meat samples collected from retail markets in north Vietnam. These rates were lower than those reported in similar previous studies in south Vietnam (Phan et al., 2005; Van et al., 2007), Thailand (Padungtod P. and Kaneene J.B., 2006) and Portugal (Antunes et al., 2003). However, these rates were higher than those in Denmark (Skov et al., 2007) and Turkey (Arslan and Eyi, 2010). Several factors must be considered when making such comparisons, including the region (different countries), source and type of meat, sampling season, slaughterhouse sanitation, and isolation method. We found 14 *Salmonella* serovars in retail meat samples, i.e. Anatum, Infantis, Emek, Derby, Rissen, Typhimurium, Reading, London, Blockey, Newport, Weltevreden, Hadar, Albany and Enteritidis. Interestingly, Infantis had not been reported in previous studies in Vietnam (Phan et al., 2005; Vo et al., 2006; Van et al., 2007), whereas it was predominantly isolated in the current investigation. This serovar was frequently found in several European countries (Van Duijkeren et al., 2002; Galanis et al., 2006), the USA (Heithoff et al., 2008) and Japan (Ishihara et al., 2009). It was suggested that Infantis may cause public health concerns worldwide (Miller et al., 2010). Anatum and Rissen were commonly isolated from the pork and chicken meat samples in this study. These serovars were common non-human serotypes found in Asia (Bangtrakulnonth et al., 2004; Galanis et al., 2006) and Vietnam (Vo et al., 2006; Van et al., 2007). Typhimurium and Derby were

commonly detected, consisted with those in the previous studies (Herikstad et al., 2002; Bangtrakulnonth et al., 2004). Emek was predominantly isolated from the chicken samples, associated with poultry originating in Vietnam (Phan et al., 2005; Vo et al., 2006). Overall, the current study indicated that the distribution of *Salmonella* serovars in Vietnam was similar to that in other Asian countries (Bangtrakulnonth et al., 2004; Galanis et al., 2006). Serovars Hadar, Weltevrenden, London and Albany have been more commonly isolated in European countries (Van Duijkeren et al., 2002; Galanis et al., 2006) and the USA (Herikstad et al., 2002), also detected in our research. This may suggest that the global *Salmonella* serovar distribution is changing because of globalization, international travel and the trade of food and animal products worldwide.

The highest antimicrobial resistance observed in this study was tetracycline (58.5%), which is one of the most widely antimicrobials used in human and veterinary medicine practices. Thus, this result was expected and agreed with reports from Vietnam (Van et al., 2007) and Thailand (Padungtod and Kaneene, 2006). However, the resistance rate was much higher than that in previous reports in south Vietnam (Vo et al., 2010), Spain (Carraminana et al., 2004) and China (Yan et al., 2010). The resistance of *Salmonella* isolates to nalidixic acid, trimethoprim, chloramphenicol, ampicillin, streptomycin and sulphonamides ranged from 27.8% - 58.1%. These findings were comparable to those in previous reports in Vietnam (Van et al., 2007; Vo et al., 2010) and other countries (Poppe et al., 2001; Benacer et al., 2010; Yang et al., 2010; Wannaprasat et al., 2011). These drugs have been commonly used for treatment of *Salmonella* infection in both human and animal in these countries because the replacement of these antimicrobials to the new generation is not easy due to the high cost (Wannaprasat et al., 2011; Van et al., 2012). Nalidixic acid resistance was predominantly observed in chicken *Salmonella* isolates in this study, similar to that in previous studies in Portugal (Antunes et al., 2003), Thailand (Padungtod and Kaneene, 2006)

and China (Yan et al., 2010). In contrast, resistance to this antimicrobial by *Salmonella* isolates from several sources were absent in the USA (Chen et al., 2004) and the UK (Wilson, 2004) or at very low rates in Spain (Carraminana et al., 2004) and Japan (Ishihara et al., 2009). The high antimicrobial resistance rates found in this study may be due to the widespread application of antimicrobials to animals in Vietnam. Besides, antimicrobials can be freely purchased in veterinary drug stores, and farmers intensively use antimicrobials as prophylactics for their animals (Van Nhiem et al, 2006).

The increasing resistance of *Salmonella* to the common antimicrobials has been leading to the use of third-generation cephalosporins in order to combat salmonellosis. In this study, none of *Salmonella* isolates resisted to ceftazidime, similar to the previous report in south Vietnam (Vo et al., 2010). However, other reports described the reduction susceptibility to this antimicrobial in *Salmonella* strains from food products, veterinary and human sources (Bouchrif et al., 2009; Yan et al., 2010). The *Salmonella* isolates had low resistance rates to amoxicillin-clavulanic acid (2.1%) and norfloxacin (1.2%). These findings were in accordance with those in other reports from Vietnam (Vo et al., 2010) and the UK (Wilson, 2004). In contrast, these antimicrobials were resisted in higher level by *Salmonella* isolated from retail meats in Turkey (Arslan and Eyi., 2010) and China (Yan et al., 2010). The ciprofloxacin-resistant isolates in this study also were MDR isolates, as reported in previous studies (Vo et al, 2006; Cui et al., 2008). Although resistance to quinolones have remained rare in *Salmonella*, reduction susceptibility is occurring worldwide that will pose an enormous challenge in the treatment of *Salmonella* infections in humans and animals (Giraud et al., 2006; Strahilevitz et al., 2009).

Multidrug resistance to more than six antimicrobials in chicken *Salmonella* isolates (n = 35) was higher than that in pork *Salmonella* isolates (n = 21) ($P < 0.05$). Furthermore, we found that 14.9% (n = 36) of the isolates were resistant to 7 - 9

antimicrobials, while 8.3% (n = 20) of the isolates were resistant to 10 - 13 antimicrobials. This finding was in good agreement with those reported in a previous study in Vietnam (Van et al., 2007; Vo et al., 2010) and China (Yan et al., 2010). In this study, the serovars Infantis, Typhimurium and Anatum had high resistance rates to tetracycline, sulphonamides, streptomycin, ampicillin, chloramphenicol, trimethoprim and nalidixic acid and high rates of MDR, observed in previous reports from Vietnam (Vo et al., 2006; Van et al., 2007). The other serovars London, Blockey, Weltevreden and Reading were more susceptible to the antimicrobials tested. Due to the limited number of the *Salmonella* isolates, it is difficult to assess evidently the relationship between serovars and MDR patterns.

Fourteen (*bla*$_{TEM}$, *bla*$_{OXA-1}$, *bla*$_{PSE-1}$, *aadA1*, *sul1*, *tetA*, *tetB*, *tetG*, *cmlA1*, *floR*, *dfrA1*, *dfrA12*, *aac(3)-IV* and *aphA1-1AB*) of 17 resistance genes were detected from the resistant *Salmonella* isolates. In most cases, resistance genes were detected where the corresponding individual resistance phenotypes were observed, suggesting the expression of the genes present. High prevalence of the *bla*$_{TEM}$, *floR*, *sul1* and *dfrA1* genes were observed from the antimicrobial-resistant isolates, occurred in previous report in Germany (Miko et al., 2005). In addition, the tetracycline-resistant isolates were mediated mainly by *tetA*, consisted with other reports (Aarestrup et al., 2003; Miko et al., 2005). In this study, the mechanism for kanamycin resistance in the *Salmonella* isolates was mainly encoded by the *aphA1-IAB* gene. However, this gene was low frequency in the resistant strains of *S.* Infantis isolated from chicken in Japan (Shahada et al., 2006).

To date, epidemiological data relating to PMQRs and QRDR in *Salmonella* have been limited in Vietnam. In this study, PMQRs such as *qnrA*, *qnrB*, *qnrS*, *qepA* and *acc(6')-1b-cr* were not detected from quilonone-resistant isolates, consisted with those in previous report from Korea (Kim et al., 2011). However, PMQRs were detected in very low rate from

quinolone-resistant *Salmonella* in France (Cattoir et al., 2007), Japan (Asai et al., 2010) and the USA (Gay et al., 2006). The substitutions in the codons of *gyrA* such as Gly81, Ser83 and Asp87 in *Salmonella* were frequently observed when their MIC levels of nalidixic acid ≥128 µl/ml (Fàbrega et al., 2009; Kim et al., 2011). However, no substitutions at codons Gly81 and Asp87 in QRDR of *gyrA* were identified, but the single mutation at codon Ser83 (ser83phe) was frequently detected from quinolone-resistant isolates in this study. In addition, all of the quinolone-resistant isolates in this study showed multidrug resistance and carried multiple resistance genes. This was also frequently observed in the *Salmonella* isolates from food stuffs Vietnam (Vo et al., 2010) and other Asian countries (Wannaprasat et al., 2011; Van et al., 2012), may result in failure in salmonellosis treatment in these countries.

Our results showed the high prevalence of antimicrobial resistance and the high rate of MDR among *Salmonella* isolated from retail meat and the correlation between the genes and corresponding individual resistance phenotypes. In addition, the data described the chromosomal mutations in nalidixic acid-resistant isolates that may have resulted from the use of quinolones in animal husbandry. Therefore, some management strategies are requested to prevent resistant bacterial pathogens from the food chain in Vietnam.

3. Chapter II: Antimicrobial resistance of *Salmonella* serovars isolated from beef at retail markets in the north Vietnam

Abstract

Approximately 39.9% (63/158) of beef samples collected from retail markets in Hanoi from January to June 2009 were *Salmonella*-positive. Nine *Salmonella* serovars, Anatum (28.6%), Rissen (25.4%), Weltevreden (12.7%), Typhimurium (7.9%), Derby (7.9%), Lexington (7.9%), Dublin (4.6%), Newport (3.2%) and London (1.8%), were identified. Thirty-seven (58.7%) of the 63 *Salmonella* isolates were resistant to at least one antimicrobial tested, of which 29 (46%) isolates showed multidrug resistance (MDR). The isolates were commonly resistant to tetracycline (46.0%), sulphonamide (39.7%), ampicilline (31.7%), streptomycin (30.2%), trimethoprim (28.6%), kanamycin (28.6%) and chloramphenicol (22.2%). Fourteen [bla_{TEM}, bla_{OXA-1}, *aadA1*, *aadA2*, *sul1*, *tetA*, *tetB*, *tetG*, *cmlA1*, *floR*, *dfrA1*, *dfrA12*, *aac(3)-IV* and *aphA1-1AB*] out of 22 antimicrobial resistance genes were detected by PCR from the resistant isolates. The *catA1*, *Kn*, bla_{PSE-1} genes and plasmid-mediated quinolone resistance (PMQR) genes such as *qnrA*, *qnrB*, *qnrS*, *qepA* and *acc(6')-ib-cr* were not detected. Mutations in the *gyrA* gene leading to the amino acid changes Ser83Phe and/or Asp87Asn were found in 6 out of the 11 quinolone-resistant isolates. The data revealed that multidrug resistant *Salmonella* strains were widely distributed in the north Vietnam via the food chain and might contain multiple genes specifying identical resistant phenotypes. Thus, continuous studies are necessary to clarify the mechanisms of MDR in *Salmonella* and its spread in the livestock market.

3. 1. Introduction

Food-borne diseases caused by non-typhoid *Salmonella* represent an important public health problem and an economic burden in many parts of the world. Non-typhoidal *Salmonella* spp. are zoonotic agents and foods of animal origin are the main sources for non-typhoidal *Salmonella* spp. transmission.

Salmonella is not only a public health concern due to the cases of foodborn diseases, but also many strains are multi-drugs resistant. Resistance to antimicrobials in *Salmonella* isolates can be the result of antimicrobial misuse in both human and veterinary medicine (Pappaioanou, 2004). Several lines of evidence indicate that antimicrobial resistance among human *Salmonella* infections results from the use of antimicrobial agents in food animals (Angulo et al., 2000).

The increasing resistance of *Salmonella* to the older generation antimicrobials has been leading to the use of quinolones in order to combat salmonellosis. Although resistance to quinolones have remained rare in *Salmonella*, however, many researches described the increasing resistance to these antimicrobial groups around the world (Strahilevitz et al., 2009; Yan et al., 2010). To date, epidemiological data relating quinolones resistance mechanism in *Salmonella* have been limited in Vietnam. Moreover, almost the studies focused on the prevalence and molecular mechanisms of antimicrobial resistance of *Salmonella* spp. isolates from human medicine, whereas similar studies on *Salmonella* originating from food animals are rare and limited (Van et al., 2007; Vo et al., 2010).

In addition, in our best knowledge, chicken and pig are usually raised in the industry farms in Vietnam where they were given antimicrobials for growth promotion, prevention and treatment many times in their life. Whereas, cattle is usually raised from one to three heads per households and they were only received antimicrobials when they were sick. This situation may create the difference in antimicrobial resistance of *Salmonella* isolated from beef at the meat shops.

The aims of this study were to determine *Salmonella* contamination rate and to characterize antimicrobial resistance, antimicrobial resistance mechanism of the resistant *Salmonella* strains isolated from retail beef at meat shops in the north Vietnam.

3. 2. Materials and method

3. 2. 1. Sampling methods

A total of 158 raw beef samples were collected from 21 retail markets in Hanoi, Vietnam, from January to June 2009. In each meat shop, one sample from a carcass (>100 g) was collected and placed in a sterile plastic sampling bag and chilled in an ice box during transport to the laboratory at the Department of Microbiology - Infectious Disease - Pathology, Faculty of Veterinary Medicine, Hanoi University of Agriculture. All samples were analyzed on the day of arrival.

3. 2. 2. Salmonella isolation and serotyping

Salmonella were isolated according to the Standard ISO-6579 method (International Organization for Standardization, 1993) with some modifications. *Salmonella* isolation was described in the Chapter I. Typical *Salmonella* isolates were serotyped by slide and microtitre agglutination for O and H antigens (Difco Laboratories, Detroit, MI, USA) according to the version of the Kauffmann and White scheme (Grimont and Weill., 2007) by Department of Veterinary hygiene, National institute of veterinary research, Vietnam.

3. 2. 3. Antimicrobial susceptibility testing

The antimicrobial susceptibility of isolates was determined according to the guidelines of the Clinical and Laboratory Standards Institute, 2006. Disk diffusion assays were performed on Muller-Hinton agar with disks containing 15 different antimicrobial agents (Oxoid, UK). Except for colistin (Co, 10 μg), 14 other antimicrobials and their interpretive categories susceptible, intermediate and resistance were described in Chapter I. For colistin, the zone criteria of ≤11 mm for resistance and ≥14 mm for susceptible were used (Gales et al., 2001). An isolate was defined

"resistance" after confirmation of resistance to at least one agent tested, while "multiple resistance" was defined as resistance to three or more agents.

3.2.4. Detection of resistance genes

DNA templates used for polymerase chain reaction (PCR) were prepared by boiling bacterial cultures (Shahada et al., 2006). Detection of plasmid-mediated quinolones resistance (PMQR) genes and antimicrobial resistance genes was performed by PCR. The primer sets and the assay conditions used for amplification were listed in the Appendix 2. PCR amplification reaction mixture, analyzation of the PCR products and sequencing of quinolone resistance determining regions (QRDRs) in *gyrA* were described in the Chapter I.

3. 3. Results

Salmonella isolation and distribution: Approximately 39.9% (63/158) of beef samples collected from the retail markets in Hanoi City were *Salmonella*-positive; and 9 serovars were identified (Table 7). The common serovars were Anatum (28.6%), Rissen (25.4%), Weltevreden (12.7%), Typhimurium (7.9%), Derby (7.9%) and Lexington (7.9%), and the remaining serovars were London, Newport and Dublin (ranging from 1.6 to 4.8%).

Antimicrobial susceptibility: The *Salmonella* isolates were commonly resistant to tetracycline (46.0%), sulphonamides (39.7%), ampicillin (31.7%), streptomycin (30.2%), trimethoprim (28.6%), kanamycin (28.6%) and chloramphenicol (22.2%). None of the *Salmonella* isolates showed resistance to ceftazidime and colistin (Table 7). Thirty-seven (58.7%) of the 63 *Salmonella* isolates were resistant to at least one antimicrobial (Table 8). Twenty nine (46%) out of the 63 isolates showed multidrug resistance (MDR). MDR was frequently observed in serovars Anatum, Typhimurium, Rissen and Derby.

Detection of antimicrobial resistance genes: Fourteen (bla_{TEM}, bla_{OXA-1}, *aadA1*, *aadA2, sul1, tetA*, *tetB, tetG, cmlA1, floR, dfrA1, dfrA12, aac(3)-IV* and *aphA1-1AB*) of 17 resistance genes were detected from the resistant isolates (Table 9). Among the 29

tetracycline-resistant isolates, the *tetA* gene was detected from 16 (55.2%), while *tetB* or *tetG* genes were detected only in one (3.4%) isolate. None of the 20 ampicillin-resistant isolates carried the bla_{PSE-1} gene; the bla_{TEM} gene was detected in 18 (90.0%) isolates, of which one contained more bla_{OXA-1} gene. The *sul1* gene was detected in 20 (80%) of the 25 sulphonamide-resistant isolates. Among the 14 chloramphenicol-resistant isolates, none of the isolates contained the *catA1* gene; *floR* and *cmlA1* genes were detected in 8 (57.1%) and 7 (50%) isolates, respectively. The *aadA1* gene was found in 15 (78.9%) of the 19 streptomycin-resistant isolates, of which one contained more *aadA2* gene. Among the 18 trimethoprim-resistant isolates, 10 *dfrA1* (55.6%) and 6 *dfrA12* (33.3%) genes were displayed. All of the 18 kanmycin-resistant isolates carried the *aphA1-1AB* gene. Eight (88.9%) of the 9 gentamicin-resistant isolates contained the *aac(3)-IV* gene.

Detection of the PMQR genes and substitutions in gyrA of the quinolone-resistant isolates: PMQR genes such as *qnrA*, *qnrB*, *qnrS*, *qepA* and *acc(6')-ib-cr* were not detected from 11 quinolone-resistant isolates. Sequence analysis of the *gyrA* revealed that 6 isolates had substitutions (Table 10). In these isolates, three *S.* Typhimurium and two *S.* Anatum isolates had single substitution at *Ser83* and one *S.* Typhimurium isolate had double substitutions at *Ser83* and *Asp87*. Substitutions were found in the codon *TCC (Ser)* at position 83 to *TTC (Phe)* and in the codon *GAC (Asp)* at position 87 to *AAC (Asn)*.

3. 4. Discussion

Approximately 39.9% of beef samples were contaminated with *Salmonella*. This rate was lower than that reported in similar previous studies in south Vietnam (Phan et al., 2005; Van et al., 2007). The high levels of contamination indicate a potential breakdown of hygiene at various stages of the food processing and distribution chain and/or a lack of refrigeration of meat in Vietnam. Among the *Salmonella* isolates identified, *S.* Anatum was commonly detected in this study, similar to previously reports in Vietnam (Vo et al., 2006; Vo et al., 2010). This serovar was usually listed in the common serovars from food sources in other studies

(Bangtrakulnonth et al., 2004; Chen et al., 2004; CDC, 2006). *S.* Rissen, one of the most commonly detected serovars from both human and nonhuman sources in Asia (Bangtrakulnonth et al., 2004), was frequently recovered in our study. In addition, the serovars Typhimurium, Derby, Weltevreden and Newport were also detected in this study; these serovars were previously associated with human foodborne gastroenteritis (Aarestrup et al., 2003; Bangtrakulnonth et al., 2004; Galanis et al., 2006; CDC, 2008; Irvine et al., 2009). Therefore, foodborne diseases may occur in Vietnam because non-typhoidal *Salmonella* spp. are zoonotic agents and animal products originating are the main sources of *Salmonella* spp. transmission.

In this study, 58.7% of *Salmonella* isolates were resistant to at least one antimicrobial. Resistance to tetracycline, sulphonamide, ampicillin, streptomycin, trimethoprim and chloramphenicol was commonly observed in the *Salmonella* isolates, as shown in previous reports from Vietnam (Van et al., 2007; Ogasawa et al., 2008; Vo et al., 2010) and other Asia countries (Yang et al., 2010; Thong and Modarressi, 2011). These antimicrobials are commonly used in animal husbandry in these countries, and the increasing and inappropriate use of antimicrobials in animal farming may be the reason for these high levels of resistance (Van et al., 2007). MDR was frequently observed in the *Salmonella* isolates in this study, as shown in previous studies from Vietnam (Van et al., 2007; Ogasawa et al., 2008; Vo et al., 2010), China (Yang et al., 2010) and Malaysia (Thong and Modarressi, 2011). This might lead to human infections with foodborne antimicrobial-resistant bacteria. Therefore, it may create an enormous challenge to treatment of *Salmonella* infection in humans and animals in these countries.

All of the antimicrobial-resistant isolates were investigated for the corresponding resistance genes. High prevalence of the *tetA,* bla_{TEM}, *floR*, *sul1* and *dfrA1* genes were detected from the antimicrobial-resistant isolates, as shown in previous reports (Miko et al., 2005; Michael et al., 2006). The streptomycin resistance was mainly encoded by *aadA1* in

this study, as observed in reports from Thailand (Aarestrup et al., 2003; Chuanchuen et al., 2009). These resistance genes were also detected from integrons of resistant *Salmonella* isolates from foodstuffs in southern Vietnam (Van et al., 2007; Vo et al., 2010). All of the kanamycin-resistant isolates contained the *aphA1-1AB* gene, similar to previous reports (Frana et al., 2001; Miko et al., 2005). However, it was detected at a low rate from *S.* Infantis in Japan (Shahada et al., 2006). In this study, the resistance genes were found at high rates and were widespread in different serovars from the resistant isolates, indicating that these genes play an important role in prevalence of antimicrobial resistance among the *Salmonella* isolates from retail meats in Vietnam.

The resistance to nalidixic acid (17.5%) and ciprofloxacin (3.2%) and decreasing susceptibility to norfloxacin (3.2%) of the isolates in this study were similar to the results of a report in south Vietnam (Vo et al., 2010). In addition, different levels of resistance of *Salmonella* to these antimicrobials were also described in several countries (Eaves et al., 2004; Strahilevitz et al., 2009; Yang et al., 2010; Thong and Modarressi, 2011). This is a worldwide concern because ciprofloxacin is the drug of choice for treatment of human *Salmonella* infection. Similar to other studies (Eaves et al., 2004; Vo et al., 2010), we detected substitutions at codons *Ser83* and *Asp87* of *gyrA* from six quinolone-resistant *Salmonella* isolates. Substitutions in the codons of *gyrA* such as *Gly81*, *Ser83* and *Asp87* in *Salmonella* were frequently observed when the MIC levels of nalidixic acid and/or ciprofloxacin were very high (≥128 µl/ml for nalidixic acid and ≥ 0.25 µl/ml for ciprofloxacin) (Eaves et al., 2004; Malorny et al., 2003; Kim et al., 2011). Thus, the absence of substitutions in *gyrA* in five nalidixic acid-resistant isolates in this study may be explained by other resistance mechanisms such as decreased permeability of the outer membrane, mutations in the *gyrB, parC*, *parE* genes. These will be warranted in the further our investigation. PMQR genes were not detected in quinolone-resistant isolates in this study,

nevertheless, these genes were confirmed to play a role in the spread of quinolone-resistant pathogens through the food chain in Vietnam and other countries (Minh Vien et al., 2009; Cattoir et al., 2007; Gay et al., 2006; Strahilevitz et al., 2009). Therefore, continuous research is needed to detect the PMQR genes in *Salmonella* spp. from food sources in Vietnam.

This study indicated a high frequency of antimicrobial resistance among the *Salmonella* isolates from beef at retail markets in the north Vietnam. Resistance genes were widespread in *Salmonella* serovars isolates. Therefore, some management strategies are needed for public health to prevent foodborne diseases caused by MDR *Salmonella* from the food supply.

4. Chapter III: Antimicrobial resistance in *Salmonella* serovars isolated from circumstances of meat shops at retail markets in the north Vietnam

Abstract

A total of 97 out of 245 carcass, sewage effluent, and table surface samples in meat shops at the retail markets in the north Vietnam showed *Salmonella*-positive. Eleven *Salmonella* serovars, including Infantis, Anatum, Rissen, Reading, London, Typhimurium, Enteritidis, Agona, Newport, Emek, and Derby, were identified. The *Salmonella* isolates were tested for antimicrobial susceptibility and further investigated for antimicrobial resistance genes. Resistance to kanamycin, gentamicin, neomycin, nalidixic acid, chloramphenicol, trimethoprim, streptomycin, tetracycline, ampicillin, and sulphonamides was found in 28.9-56.7%. The isolates were neither resistant to ceftazidime nor norfloxacin. Sixty-four (66.0%) out of 97 isolates were resistant to at least one of 14 antimicrobials, and 55 (85.9%) out of the 64 isolates showed multidrug resistance. Thirteen resistance genes [bla_{TEM}, bla_{OXA-1}, *aadA1*, *sul1*, *tetA*, *tetB*, *tetG*, *cmlA1*, *floR*, *dfrA1*, *dfrA12*, *aac(3)-IV*, and *aphA1-1AB*] were detected in the resistant isolates. This study indicates that *Salmonella* isolated from meat shops were resistant to multiple antimicrobials, and the resistance genes were widespread among the serovars isolated.

4. 1. Introduction

Foodborne diseases caused by non-typhoid *Salmonella* represent an important public health concern and an economic burden in many parts of the world (Chen et al., 2004; Miko et al., 2005; Van et al., 2007). In recent years, an increase in the occurrence of antimicrobial resistance *Salmonella* spp. has been observed in several countries (Van et al., 2007; Yang et al., 2010). Antimicrobials are currently used for three main reasons in animal husbandry such as therapeutics, prophylaxis and growth promotion. The routine practice of giving antimicrobials could be important factors in the emergence of antimicrobial-resistant bacteria that are subsequently transferred to humans via the food chain (Tollefson and Miller, 2000; Angulo et al., 2004). Several lines of evidence indicate that antimicrobial resistance among human *Salmonella* infections results from the use of antimicrobials in food animals (Angulo et al., 2000).

In Vietnam, as in most other developing countries, the information of foodborne diseases and antimicrobial resistance among *Salmonella* has been restricted. In addition, self-medication through retail pharmacies is a common practice, where antimicrobials for human and animal can be freely purchased over the counter without control (Ogasawara et al., 2008). To date, most of the studies focused on the prevalence and molecular mechanisms of antimicrobial resistance of *Salmonella* spp. isolates from human medicine, whereas similar studies on *Salmonella* originating from foodstuffs are rare and limited (Van et al., 2007; Vo et al., 2010).

The aim of this study was to determine the level of antimicrobial resistance and to investigate the antimicrobial resistance genes in *Salmonella* serovars isolated from meat shops at retail markets in the north Vietnam.

4. 2. Materials and methods

4. 2. 1. Sampling and Salmonella isolation

A total of 245 samples (116 from carcass, 84 table surfaces and 45 sewages) were collected in the 200 pork and chicken meat shops at 45 retail markets in the north Vietnam between January 2008 and June 2009. Only one kind of the samples per each meat shop was collected. Swab of carcass and table surfaces were sampled by autoclaved cottons in an area approximately 20 cm^2 and placed in sterile bags with 90-ml buffered peptone water. In each retail market, approximately 100 ml of sewages was taken to 200 ml bottle and then 10 ml was mixed with 90-ml buffered peptone water for pre-enrichment. All samples were transported and examined in the laboratory of Department of microbiology, infectious disease and pathology, Faculty of veterinary medicine, Hanoi University of agriculture, Vietnam. The isolation methods of *Salmonella* were previously described (Vo et al., 2006; Van et al., 2007). Typical *Salmonella* isolates were serotyped by slide and microtitre agglutination for O and H antigens (Difco Laboratories, Detroit, MI, USA) according to the Kauffmann and White scheme (Grimont and Weill, 2007) by Department of Veterinary hygiene, National institute of veterinary research, Vietnam.

4. 2. 2. Antimicrobial susceptibility testing

The antimicrobial susceptibility of isolates was determined according to the guidelines of the Clinical and Laboratory Standards Institute 2006. Disk diffusion assays were performed on Muller-Hinton agar with disks containing 14 different antimicrobial agents (Oxoid, UK). The antimicrobial agents and the interpretive categories susceptible, intermediate or resistance were described in the Chapter I.

4. 2. 3. Detection of resistance genes

DNA templates used for polymerase chain reaction (PCR) were prepared by boiling bacterial cultures (Shahada et al., 2006). Detection of antimicrobial resistance genes was

performed by PCR. The primer sets and the assay conditions used for amplification were listed in the Appendix 2. PCR amplification reaction mixture and analyzation of the PCR products were described in the Chapter I.

4. 2. 4. Data and statistical analysis

Statistical comparison of the prevalence of *Salmonella*, the rate of antimicrobial resistance from different sources was analyzed by the Chi-square test (Microsoft Excel 2003).

4. 3. Results

Distribution of Salmonella serovars: Approximately 39.6% (97/245) of the samples were contaminated with *Salmonella,* of which 44.0% (51/116) from carcass, 35.7% (30/84) from table surface, and 35.5% (16/45) from sewage samples; and 11 serovars were identified (Table 11). The rate of *Salmonella* in three kinds of samples was not significantly different ($P > 0.05$). *S.* Infantis (33.0%) was the most common serovar, followed by Anatum (15.5%), Rissen (12.4%), and Reading (11.3%). *S.* Typhimurium and Enteritidis were detected in 4.1% and 3.1% of the *Salmonella* isolates. Others serovars such as Emek, Derby, Newport, Agona and London ranged from 3.1 to 6.2%.

Antimicrobial resistance: The prevalence of antimicrobial resistance of the *Salmonella* isolates was shown in Table 12. There was no significant difference between the resistance rates of 14 antimicrobials among 3 kinds of samples ($P > 0.05$). The highest resistance was sulphonamides (56.7%), followed by ampicillin and tetracycline (48.5%), streptomycin (44.3%), trimethoprim (43.3%), chloramphenicol (42.3%) and nalidixic acid (40.2%). Resistance to gentamicin, neomycin and kanamycin ranged from 28.9% to 38.1%. None of the isolates were resistant to ceftazidime and norfloxacin. Only 4 (4.1%) and 6 (6.2%) of the isolates were resistant to amoxicillin-clavunalic acid and ciprofloxacin, respectively.

Resistance to at least one antimicrobial was found in 64 (66.0%) isolates (Table 13). Fifty five (85.9%) out of the 64 antimicrobial-resistant isolates showed multidrug resistance

(MDR). Of which, 20 isolates (31.3%) were resistant to 9 - 11 antimicrobials. MDR was widespread among the *Salmonella* serovars, and found frequently in the serovars Typhimurium, Infantis, Anatum, and Rissen.

The distribution and prevalence of resistance genes: The distribution and prevalence of resistance genes among the *Salmonella* serovars were shown in Table 14. Thirteen (bla_{TEM}, bla_{OXA-1}, *aadA1*, *sul1, tetA*, *tetB, tetG, cmlA1, floR, dfrA1, dfrA12, aac(3)-IV* and *aphA1-1ab*) out of 17 investigated resistance genes were detected. Among the 51 ampicillin-resistant isolates, the bla_{TEM} and bla_{OXA-1} genes were detected in 38 (74.5%) and 8 (15.7%) isolates, respectively. Of the 50 tetracycline-resistant isolates, 37 (74.0%) were positive for *tetA*, 13 (26.0%) for *tetG* and 3 (6.0%) for *tetB;* of which, 8 (16.0%) carried both the *tetA* and *tetG* genes. The *sul1* gene was detected in 52 (89.7%) out of the 58 sulphonamides-resistant isolates. No *aadA2* gene was found in the 45 streptomycin-resistant isolates; instead, *aadA1* gene was found in 44 (97.8%) of the resistant isolates. Among the 41 kanamycin-resistant isolates, 39 (95.1%) were positive for the *aphA1-Iab* gene. Of the 30 gentamicin-resistant isolates, 27 (90.0%) carried the *aac(3)-IV* gene. The *catA1* gene was not detected in the 47 chloramphenicol-resistant isolates, whereas the *cmlA1* and *floR* genes were detected in 29 (61.7%) and 36 (76.6%) of the isolates, respectively. Among the 45 trimethoprim-resistant isolates, 39 (86.7%) and 9 (20%) of the isolates were positive for *dfrA1* and *dfrA12*, respectively.

4. 4. Discussion

In this study, eleven *Salmonella* serovars were identified. Interestingly, although *S.* Infantis was not reported in previous studies in Vietnam (Vo et al., 2006; Van et al., 2007), it was the most common serovar in this study. This serovar was detected in several European countries (Galanis et al., 2006; Miller et al., 2010), and the USA (Heithoff el al., 2008). It suggests that *S.* Infantis may cause public health concerns worldwide. We observed that *S.*

Rissen and Anatum were frequently detected from the pork and chicken meat shops. These serovars have been reported in both human and non-human sources in Asia (Bangtrakulnonth et al., 2004; Galanis et al., 2006) and Vietnam (Vo et al., 2006; Van et al., 2007). In addition, the serovars Typhimurium, Enteritidis, Derby and Newport, which were previously associated with human foodborne disease in Asian countries (Bangtrakulnonth et al., 2004; Galanis et al., 2006) and in the USA (CCD, 2008), were also detected in this study. Therefore, they may create the public health concerns in Vietnam as non-typhoidal *Salmonella* spp. is zoonotic agent and could be transmitted through foods of animal origin.

This study demonstrated the high incidence of resistance to sulphonamides, tetracycline, ampicillin, chloramphenicol, streptomycin and trimethoprim in the *Salmonella* isolates. These findings were comparable to those in previous reports from Vietnam (Van et al., 2007; Vo et al., 2010), China (Yan et al., 2010; Yang et al., 2010) and Thailand (Wannaprasat et al., 2011). The high resistance to nalidixic acid in the *Salmonella* isolates observed in this study consisted with that in Thailand (Padungtod and Kaneene, 2006) and Vietnam (Van et al., 2007; Vo et al., 2010). This could suggest that these antimicrobials are widely used in animal husbandry in many countries. The resistance rates in the *Salmonella* isolates against gentamicin and kanamicin in this study was higher than those in other Asian countries (Benacer et al., 2010; Wannaprasat et al., 2011). The explanation may be these antimicrobials were more frequently used in animal husbandry in recent years after other antimicrobials were not allowed use in this field (Ministry of Agriculture and Rural Development, Vietnam, 2009). In this study, there were no *Salmonella* isolates resistant to ceftazidime, similar to the previous report in south Vietnam (Vo et al., 2010). However, other reports described the reduction susceptibility to this antimicrobial in *Salmonella* strains from food products in China (Yang et al., 2010), from veterinary and human sources in Morocco (Bouchrif et al., 2009). Moreover, multidrug-resistant *Salmonella* isolates was also

found frequently in this study, similar to that in the south Vietnam (Van et al., 2007; Vo et al., 2010), China (Yan et al., 2010; Yang et al., 2010) and Thailand (Wannaprasat et al., 2011) so far. Hence, it may create challenges to the treatment of *Salmonella* infections in humans and animals.

In this study, the *sul1* gene was commonly present in the sulphonamides-resistant isolates, occurred in previous reports from Germany (Miko et al., 2005). The genes encoding for resistance to ampicillin (bla_{TEM}) and streptomycin (*aadA1)* were frequently detected in the isolates, observed in previous reports (Chen et al., 2004; Miko et al., 2005, Shahada et al., 2006). Similar to the previous studies (Hamada et al., 2005; Miko et al., 2005), mechanism for kanamycin resistance in the *Salmonella* isolates was mainly encoded by *aphA1-IAB* gene. However, the frequency of this gene was lower in the resistant strains of *S.* Infantis isolated from chicken in Japan (Shahada et al., 2006). Our results showed that resistance to gentamycin and trimethoprim were mainly mediated by the *aac(3)-IV* and *dfrA1* genes. These genes may play important role for resistance to these antimicrobials of *Salmonella* in Vietnam. The *cmlA1* and *floR* genes were frequently detected in the chloramphenicol-resistant isolates. However, the molecular study of chloramphenicol resistance has received little attention so far, due to the limited usage in human and ban in animal husbandry (Fluit et al., 2001). In this study, resistance to tetracycline was mainly mediated by the *tetA* gene, consisted with that in previous reports (Asai et al., 2006; Chuanchuen and Padungtod., 2009). These findings may not be surprising even though *Salmonella* isolates are often found to carry *tetA, tetB, tetC, tetD* and *tetE* genes (Michael et al., 2006).

In summary, *Salmonella* contamination was common at retail meat shops in the north Vietnam. The *Salmonella* isolates were resistant to multiple antimicrobials, and resistance genes were widespread among the serovars isolated. This problem should be closely monitored to minimize further health impacts in Vietnam.

5. Conclusion

The prevalence of *Salmonella* contamination at the retail markets in the north Vietnam is high. These indicate a potential breakdown of hygiene at various stages of the food processing and distribution chain and/or a lack of refrigeration of meat in Vietnam. Seventeen *Salmonella* serovars including Infantis, Anatum, Rissen, Reading, Emek, Typhimurium, Blockey, London, Newport, Derby, Weltevreden, Anbany, Hadar, Agona, Lexington and Dublin were determined. In overall, the distribution of *Salmonella* serovars was similar to that from previous reports in Vietnam and other Asian countries.

The *Salmonella* serovars isolated from retail meat samples were resistant to multiple antimicrobials and this resistance was widespread among different serovars. The widespread resistance may link to the widespread of antimicrobial use in animal husbandry in northern Vietnam. Moreover, antimicrobial resistance was frequently found in the serovars of epidemiological importance such as Typhimurium, Infantis, Anatum, Rissen and Emek. Therefore, resistance to these antimicrobials in the *Salmonella* serovars may create problems for disease treatment and should be closely monitored to minimise future health impacts in Vietnam.

The data also revealed that resistant *Salmonella* strains were widely distributed in northern Vietnam via the food chain and that they might contain multiple genes and mutations at chromosome specifying identical resistance phenotypes. Fifteen out of 17 resistance genes were detected from the antimicrobial-resistant *Salmonella* strains. Besides, substitutions at codons *Ser83Phe* and/or *Asp87Asn* of *gyrA* were frequently dectected from quinolone-resistant *Salmonella* isolates. These situations may result in failure in salmonellosis treatment in animal husbandry in Vietnam. Although, plasmid mediated quinolone resistance (PMQR) were not detected in quinolone-resistant isolates. However, these genes were confirmed to play a role in the spread of quinolone-resistant pathogens

through the food chain. Therefore, continuous research is needed to detect the PMQR genes in *Salmonella* from food sources in Vietnam.

Thus, further studies are necessary to clarify the mechanisms of antimicrobial resistance in *Salmonella* and their spread in the livestock market. The application of hygiene practices along the food chain and prudent use of antimicrobials in animal husbandry are essential and these applications may help to reduce the risks of food poisoning.

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7. Tables and appendixes

Table 1. Distribution of *Salmonella* serovars isolated from pork and chicken meat at retail markets

Serovar	Chicken no	Pork no	Total no (%)
Anatum	13	25	38 (15.8)
Infrantis	15	17	32 (13.3)
Emek	25	-	25 (10.4)
Derby	3	20	23 (9.5)
Rissen	10	13	23 (9.5)
Typhimurium	5	17	22 (9.1)
Reading	8	10	18 (7.5)
London	7	8	15 (6.2)
Blockey	14	-	14 (5.8)
Newport	3	8	11 (4.6)
Weltevreden	-	8	8 (3.3)
Hadar	5	-	5 (2.1)
Albany	4	-	4 (1.7)
Enterititis	3	-	3 (1.2)
Total	**115**	**126**	**241 (100)**

-: not found

Table 2. Number and prevalence of antimicrobial resistance of *Salmonella* serovars isolated from pork and chicken meat at retails markets

Anti-microbials	Anatum (38 isolates) no (%)	Infantis (32 isolates) no (%)	Emek (25 isolates) no (%)	Derby (23 isolates) no (%)	Rissen (23 isolates) no (%)	Typhimurium (22 isolates) no (%)	Reading (18 isolates) no (%)	London (15 isolates) no (%)	Blockey (14 isolates) no (%)	Newport (11 isolates) no (%)	Others (20 isolates) no (%)	Total (241 isolates) no (%)
A	21 (55.3)	19 (59.4)	6 (24.0)	6 (26.1)	8 (34.8)	15 (68.2)	3 (16.7)	1 (6.7)	0 (0.0)	5 (45.5)	12 (60.0)	96 (39.8)
Ac	1 (2.6)	1 (3.1)	1 (4.0)	0 (0.0)	0 (0.0)	4 (18.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	7 (2.9)
C	15 (39.5)	15 (46.9)	18 (72.0)	5 (21.7)	9 (39.1)	15 (68.2)	4 (22.2)	2 (13.3)	0 (0.0)	4 (36.4)	3 (15.0)	90 (37.3)
Cf	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Ci	2 (5.3)	3 (9.4)	1 (4.0)	1 (4.3)	0 (0.0)	5 (22.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	12 (5.0)
G	7 (18.4)	6 (18.8)	5 (20.0)	4 (17.4)	5 (21.7)	12 (54.5)	1 (5.6)	0 (0.0)	0 (0.0)	2 (18.2)	1 (5.0)	43 (17.8)
K	8 (21.1)	19 (59.4)	5 (20.0)	1 (4.3)	9 (39.1)	9 (40.9)	1 (5.6)	1 (6.7)	0 (0.0)	4 (36.4)	2 (10.0)	59 (24.5)
Na	10 (26.3)	14 (43.8)	5 (20.0)	4 (17.4)	6 (26.1)	17 (77.3)	4 (22.2)	0 (0.0)	0 (0.0)	3 (27.3)	4 (20.0)	67 (27.8)
Ne	5 (13.2)	9 (28.1)	4 (16.0)	1 (4.3)	4 (17.4)	9 (40.9)	0 (0.0)	0 (0.0)	0 (0.0)	3 (27.3)	2 (10.0)	37 (15.4)
No	1 (2.6)	1 (3.1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (1.2)
Su	16 (42.1)	27 (84.4)	20 (80.0)	15 (65.2)	13 (56.5)	19 (86.4)	5 (27.8)	5 (33.3)	6 (42.9)	4 (36.4)	10 (50.0)	140 (58.1)
S	8 (21.1)	26 (81.3)	10 (40.0)	14 (60.9)	12 (52.2)	18 (81.8)	6 (33.3)	4 (26.7)	6 (42.9)	3 (27.3)	7 (35.0)	114 (47.3)
T	23 (60.5)	24 (75.0)	8 (32.0)	7 (30.4)	14 (60.9)	19 (86.4)	10 (55.6)	10 (66.7)	7 (50.0)	6 (54.5)	13 (65.0)	141 (58.5)
Tp	15 (39.5)	20 (62.5)	8 (32.0)	2 (8.7)	7 (30.4)	8 (36.4)	5 (27.8)	2 (13.3)	2 (14.3)	5 (45.5)	8 (40.0)	82 (34.0)

Abbreviation: Ampicillin (A); Amoxicillin/clavulanic acid (Ac); Chloramphenicol (C); Ceftazidime (Cf); Ciprofloxacin (Ci); Gentamicin (G); Kanamicin (K); Nalixidic acid (Na); Neomycin (Ne); Norfloxacin (No); Sulphonamides (Su); Streptomicin (S); Tetracycline (T); Trimethoprim (Tp)

Table 3. Multidrug resistance (MDR) among *Salmonella* serovars isolated from pork and chicken meat at retail markets

Serovars	no (%) of antibiotics resistance				
	1-3	**4-6**	**7-9**	**≥10**	**Total** (resistance ≥ 1)
Anatum (38)	8 (21.1)	11 (28.9)	6 (15.8)	2 (5.3)	27 (71.1)
Infrantis (32)	5 (15.6)	11 (34.4)	11 (34.4)	3 (9.4)	30 (93.8)
Emek (25)	16 (64.0)	1 (4.0)	2 (8.0)	3 (12.0)	22 (88.0)
Derby (23)	10 (43.5)	2 (8.7)	3 (13.0)	1 (4.3)	16 (69.6)
Rissen (23)	7 (30.4)	6 (26.1)	2 (8.7)	3 (13.0)	18 (78.3)
Typhimurium (22)	0 (0.0)	5 (22.7)	9 (40.9)	5 (22.7)	19 (86.4)
Reading (18)	6 (33.3)	4 (22.2)	1 (5.6)	0 (0.0)	11 (61.1)
London (15)	7 (46.7)	3 (20.0)	0 (0.0)	0 (0.0)	10 (66.7)
Blockey (14)	9 (64.3)	0 (0.0)	0 (0.0)	0 (0.0)	9 (64.3)
Newport (11)	5 (45.5)	1 (9.1)	1 (9.1)	2 (18.2)	9 (81.8)
Weltevreden (8)	3 (37.5)	3 (37.5)	1 (12.5)	0 (0.0)	7 (87.5)
Hadar (5)	4 (80.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (80.0)
Albany (4)	3 (75.0)	0 (0.0)	0 (0.0)	1 (25.0)	4 (100)
Enterititis (3)	0 (0.0)	3 (100)	0 (0.0)	0 (0.0)	3 (100)
Total (241)	83 (34.4)	50 (20.7)	36 (14.9)	20 (8.3)	189 (78.4)

Table 4. Multidrug resistance (MDR) of *Salmonella* serovars isolated from pork and chicken meat at retail markets

Model of resistance patterns	MDR of *Salmonella* serovars isolated from retail meats			No of AR
	Serovars (pork/chicken)	no (%)	MDR patterns	
1	Rissen (0/1)	1 (0.6)	ASuT	3
2	Emek (0/4)	4 (2.5)	CSSu	3
3	Infantis (1/0); Rissen (2/0)	3 (1.9)	CSuT	3
4	Emek (0/3); Reading (1/0)	4 (2.5)	CSuTp	3
5	Anatum (1/0); Newport (1/0); Reading (1/0)	3 (1.9)	CTTp	3
6	Risen (1/0)	1 (0.6)	SSuG	3
7	Blockey (0/4); Infantis (0/1); London (0/2); Reading (1/0)	8 (5.0)	SSuT	3
8	Blockey (0/2); Derby (0/1); Infantis (0/1)	4 (2.5)	SSuTp	3
9	Anatum (0/2)	2 (1.2)	ACTNa	4
10	Anatum (3/0)	3 (1.9)	ACTTp	4
11	Derby (1/0); Infantis (1/0)	2 (1.2)	ASSuT	4
12	Infrantis (0/2)	2 (1.2)	ASSuTp	4
13	Anatum (3/0)	3 (1.9)	ASuTTp	4
14	London (1/0); Reading (1/0)	2 (1.2)	CSSuT	4
15	London (1/0); Weltevreden (1/0)	2 (1.2)	CSuTTp	4
16	Rissen (2/0)	2 (1.2)	CTKNa	4
17	Infantis (2/0); Rissen (0/1)	3 (1.9)	SSuKTp	4
18	Typhimurium (3/0)	3 (1.)	SSuTNa	4
19	Reading (0/2)	2 (1.2)	STTpNa	4
20	Derby (0/1); Typhimurium (2/0); Rissen (0/1)	4 (2.5)	ACSSuT	5
21	Emek (0/1); Enterititis (0/3); Rissen (0/1)	5 (3.1)	ASSuTTp	5
22	Anatum (1/0); Weltevreden (2/0)	3 (1.9)	ASTTpNa	5
23	Anatum (1/0); Newport (0/1); Infantis (2/1); London (1/0); Rissen (1/0)	7 (4.4)	CSuTKTp	5
24	Anatum (0/1); Infantis (0/1)	2 (1.2)	ACSSuTTp	6
25	Reading (0/1)	1 (0.6)	ASSuTKNa	6
26	Infrantis (0/1)	1 (0.6)	ASSuTKTp	6
27	Infantis (1/0)	1 (0.6)	CSSuGTpNa	6
28	Infrantis (0/1); Rissen (0/1)	2 (1.2)	ACSSuTGK	7
29	Derby (3/0); Typhimurium (3/0)	6 (3.8)	ACSSuTGNa	7

30	Anatum (0/1)	1 (0.6)	ACSSuTGTp	7
31	Infrantis (0/1)	1 (0.6)	ACSSuTKNa	7
32	Weltevreden (1/0)	1 (0.6)	ACSuTKNeNa	7
33	Infantis (1/0)	1 (0.6)	ACTKNeTpNa	7
34	Reading (0/1)	1 (0.6)	ASSuTGTpNa	7
35	Infantis (2/0); Typhimurium (1/0)	3 (1.9)	ASSuTKNeNa	7
36	Infantis (1/0); Typhimurium (1/0)	2 (1.2)	CSSuTKNeNa	7
37	Infrantis (0/1); Typhimurium (0/2)	3 (1.9)	ACSSuTGTpNa	8
38	Anatum (0/1); Infantis (1/1)	3 (1.9)	ACSSuTKTpNa	8
39	Anatum (0/1); Emek (0/1)	2 (1.2)	ACSuTGKTpNa	8
40	Newport (1/0); Rissen (0/1)	2 (1.2)	ASSuTKNeTpNa	8
41	Anatum (0/2); Emek (0/1); Typhimurium (1/0)	4 (2.5)	ACSSuTGKNeNa	9
42	Infantis (2/0)	2 (1.2)	ACSSuTKNeTpNa	9
43	Anatum (0/1); Typhimurium (1/0)	2 (1.2)	ACSuTGKNeTpNa	9
44	Albany (0/1); Emek (0/2); Newport (0/2); Rissen (0/3)	8 (5.0)	ACSSuTGKNeTpNa	10
45	Anatum (0/1); Derby (0/1); Infantis (0/2); Typhimurium (0/1)	5 (3.1)	ACSSuTGKNeTpNaCi	11
46	Emek (0/1); Typhimurium (1/1); Newport (1/0)	4 (2.5)	ACSSuTGKNeTpNaCiAc	12
47	Anatum (0/1); Infantis (0/1); Typhimurium (0/1)	3 (1.9)	ACSSuTGKNeTpNaNoCiAc	13
Total		134 (100)		

Abbreviation: Ampicillin (A); Amoxicillin/clavulanic acid (Ac); Chloramphenicol (C); Ceftazidime (Cf); Ciprofloxacin (Ci); Gentamicin (G); Kanamicin (K); Nalixidic acid (Na); Neomycin (Ne); Norfloxacin (No); Sulphonamides (Su); Streptomicin (S); Tetracycline (T); Trimethoprim (Tp); AR: Antimicrobial resistance

Table 5. The distribution and prevalence of resistance genes among the *Salmonella* serovars isolated from pork and chicken meat at retail markets

AR genes (number of isolates tested)	Number of positive isolates (%)	AR genes belong to serovars (number of positive isolates)
Ampicillin (39)		
bla$_{TEM}$	34 (87.1)	Anatum (9); Derby (1), Emek (1); Infantis (7); London (1); Newport (3); Reading (1); Rissen (5); Typhimurium (6)
bla$_{OXA-1}$	9 (23.1)	Anatum (2); Derby (1); Infantis (1); Rissen (2); Typhimurium (3)
bla$_{PSE-1}$	2 (5.1)	Derby (1); Infrantis (1)
bla$_{TEM}$+*bla*$_{OXA-1}$	5 (12.8)	Anatum (2); Typhimurium (3)
bla$_{TEM}$+*bla*$_{PSE-1}$	1 (2.6)	Derby (1)
at least one gene	39 (100)	
Chloramphenicol (42)		
cmlA1	24 (57.1)	Anatum (6); Derby (1); Emek (1); Infantis (3); London (1); Newport (4); Rissen (4); Typhimurium (4)
floR	29 (69.0)	Anatum (2); Derby (2); Infantis (6), London (1), Newport (4), Reading (3), Rissen (6); Typhimurium (4); Weltevreden (1)
cmlA1+floR	14 (33.3)	Anatum (1); Derby (1); Infantis (2); Newport (3); Rissen (4); Typhimurium (3)
at least one gene	39 (92.9)	
Gentamycin (18)		
aac(3)-IV	17 (94.4)	Anatum (4); Derby (1); Infantis (2), Newport (2); Rissen (4); Typhimurium (4)
Kanamicin (31)		
aphA1-1AB	31 (100)	Anatum (5); Derby (1); Infantis (10); Newport (4); Rissen (5); Typhimurium (6)
Steptomycin (49)		
aadA1	41 (83.7)	Anatum (7); Derby (3); Emek (1); Infantis (13); Newport (3); Reading (1); Rissen (8); Typhimurium (5)
Sulphonamides (62)		
sul 1	47 (75.8)	Anatum (7); Derby (3); Emek (2); Infantis (14); London (1); Newport (3); Reading (1); Rissen (9); Typhimurium (6); Weltevreden (1)
Tetracycline (64)		
tetA	47 (73.4)	Anatum (7); Derby (1); Emek (1); Infantis (10); London (2); Newport (5); Reading (5); Rissen (9); Typhimurium (6); Weltevreden (1)
tetB	1 (1.6)	Anatum (1)
tetG	20 (31.3)	Anatum (4); Derby (1); Infantis (2); Newport (4); Reading (1); Rissen (3); Typhimurium (4); Weltevreden (1)
tetA+tetB	1 (1.6)	Anatum (1)
tetA+tetG	16 (25.0)	Anatum (2); Derby (1); Infantis (1); Newport (3); Reading (1); Rissen (3); Typhimurium (4); Weltevreden (1)
at least one gene	52 (81.3)	
Trimethoprim (43)		
dfrA1	20 (46.5)	Anatum (3); Derby (1); Emek (2); Infantis (4); London (1); Reading (2); Rissen (4); Typhimurium (2); Weltevreden (1)
dfrA12	20 (46.5)	Anatum (2); Derby (1); Infantis (6); Newport (3); Reading (1); Rissen (3); Typhimurium (4)
dfrA1+dfrA12	7 (16.3)	Anatum (1); Newport (1); Reading (1); Rissen (2); Typhimurium (2)
at least one gene	37 (86.0)	

AR: Antimicrobial resistance

Table 6. Resistance profile and characteristic of quinolone resistance in *Salmonella* serovars isolated from pork and chicken meat at retail markets

Serovars	Origins	Mutations in *gyrA*	MICs for Na (µg/ml)	Disk diffusion		Resistance phenotypes	Resistance genes
				Ci	No		
Anatum	C	WT	≥ 32	S	S	ACTNa	*bla*$_{TEM}$, *floR*
Anatum	P	WT	≥ 32	S	S	ASTTpNa	*bla*$_{TEM}$, *tetA*, *aadA1*
Infrantis	C	WT	≥ 32	S	S	ACSSuTKNa	*bla*$_{TEM}$, *floR*, *aadA1*, *sul1*, *tetA*, *aphA1-1AB*,
Infantis	P	WT	≥ 64	S	S	CSSuTKNeNa	*floR*, *sul1*, *aadA1*, *tetA*, *aphA1-1AB*
Anatum	C	Wt	≥ 64	S	S	ACSSuTKTpNa	*bla*$_{TEM}$, *cmlA1*, *aadA1*, *sul1*, *tetA*, *tetG*, *aphA1-1AB*, *dfrA1*
Infantis	P	Ser83Phe	≥ 128	S	S	CSSuGTpNa	*cmlA1*, *floR*, *aadA1*, *sul1*, *aac(3)-IV*, *dfrA12*
Typhimurium	P	Ser83Phe	≥ 128	S	S	ASSuTKNeNa	*bla*$_{TEM}$, *aadA1*, *sul1*, *tetA*, *aphA1-1AB*
Infrantis	C	Ser83Phe	≥ 128	S	S	ACSSuTKTpNa	*bla*$_{TEM}$, *cmlA1*, *aadA1*, *sul1*, *tetA*, *tetG*, *aphA1-1AB*, *dfrA12*
Newport	P	Ser83Phe	≥ 128	S	S	ACSTKNeTpNa	*bla*$_{TEM}$, *cmlA1*, *floR*, *aadA1*, *tetA*, *aphA1-1AB*, *dfrA1*
Risen	C	Ser83Phe	≥ 128	S	S	ASSuTKNeTpNa	*bla*$_{TEM}$, *aadA1*, *sul1*, *tetA*, *tetG*, *aphA1-1AB*, *dfrA1*
Anatum	C	Ser83Phe	≥ 128	S	S	ACSSuTGKNeNa	*bla*$_{TEM}$, *bla*$_{OXA-1}$, *cmlA1*, *floR*, *aadA1*, *sul1*, *tetG*, *aac(3)-IV*, *aphA1-1AB*
Anatum	C	Ser83Phe	≥ 128	S	S	ACSSuTGKNeNa	*bla*$_{TEM}$, *bla*$_{OXA-1}$, *cmlA1*, *aadA1*, *sul1*, *tetG*, *aac(3)-IV*, *aphA1-1AB*
Newport	C	Ser83Phe	≥ 256	I	S	ACSSuTGKNeTpNa	*bla*$_{TEM}$, *cmlA1*, *floR*, *aadA1*, *sul1*, *tetA*, *tetG*, *aac(3)-IV*, *aphA1-1AB*, *dfrA1*, *dfrA12*
Newport	C	Ser83Phe	≥ 256	I	S	ACSSuTGKNeTpNa	*bla*$_{TEM}$, *cmlA1*, *floR*, *aadA1*, *sul1*, *tetA*, *tetG*, *aac(3)-IV*, *aphA1-1AB*, *dfrA12*
Risen	C	Ser83Phe	≥ 256	S	S	ACSSuTGKNeTpNa	*bla*$_{TEM}$, *cmlA1*, *floR*, *aadA1*, *sul1*, *tetA*, *tetG*, *aac(3)-IV*, *aphA1-1AB*, *dfrA1*, *dfrA12*
Risen	C	Ser83Phe	≥ 512	S	I	ACSSuTGKNeTpNa	*bla*$_{TEM}$, *cmlA1*, *floR*, *aadA1*, *sul1*, *tetA*, *tetG*, *aac(3)-IV*, *aphA1-1AB*, *dfrA1*, *dfrA12*
Anatum	C	Ser83Phe	≥ 512	R	S	ACSSuTGKNeTpNaCi	*bla*$_{TEM}$, *cmlA1*, *aadA1*, *sul1*, *tetA*, *tetB*, *aac(3)-IV*, *aphA1-1AB*, *dfrA1*, *dfrA12*
Derby	C	Ser83Phe	≥ 512	R	S	ACSSuTGKNeTpNaCi	*bla*$_{TEM}$, *cmlA1*, *floR*, *aadA1*, *sul1*, *tetA*, *tetG*, *aac(3)-IV*, *aphA1-1AB*, *dfrA12*
Typhimurium	C	Ser83Phe	≥ 512	R	I	ACSSuTGKNeTpNaCi	*bla*$_{TEM}$, *cmlA1*, *aadA1*, *sul1*, *tetA*, *tetG*, *aac(3)-IV*, *aphA1-1AB*, *dfrA12*
Typhimurium	C	Ser83Phe	≥ 512	R	S	ACSSuTGKNeTPNaCiAc	*bla*$_{TEM}$, *bla*$_{OXA-1}$, *cmlA1*, *floR*, *aadA1*, *sul1*, *tetA*, *tetG*, *aac(3)-IV*, *aphA1-1AB*, *dfrA1*, *dfrA12*
Typhimurium	P	Ser83Phe	≥ 512	R	I	ACSSuTGKNeTpNaCiAc	*bla*$_{TEM}$, *bla*$_{OXA-1}$, *floR*, *sul1*, *tetA*, *tetG*, *aphA1-1AB*, *dfrA12*
Typhimurium	P	Ser83Phe	≥ 512	R	I	ACSSuTGKNeTpNaCiAc	*bla*$_{TEM}$, *cmlA1*, *floR*, *aadA1*, *sul1*, *tetA*, *aac(3)-IV*, *aphA1-1AB*, *dfrA12*
Typhimurium	C	Ser83Phe	≥ 512	R	R	ACSSuTGKNeTpNaNoCiAc	*bla*$_{TEM}$, *bla*$_{OXA-1}$, *cmlA1*, *floR*, *aadA1*, *sul1*, *tetA*, *tetB*, *tetG*, *aac(3)-IV*, *aphA1-1AB*, *dfrA1*, *dfrA12*

Abbreviation: Na: Nalidixic acid; No: Norfloxacin; Ci: Ciprofloxacin; C: chicken meat; P: pork meat; R: resistance; I: intermediate; S: susceptible

Table 7. Antimicrobial resistance of *Salmonella* serovars isolated from retail beef at retail markets

Serovars (no)	A	Ac	Cf	Co	S	G	K	C	Ne	Na	No	Ci	T	Su	Tp
Anatum (18)	7	-	-	-	6	3	8	7	3	3	-	-	8	10	8
Rissen (16)	5	-	-	-	3	1	3	-	1	2	-	-	10	4	4
Weltevreden (8)	1	-	-	-	1	-	-	-	-	-	-	-	1	1	-
Typhimurium (5)	5	-	-	-	4	4	4	4	1	4	-	2	5	5	4
Lexington (5)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Derby (5)	2	-	-	-	3	-	1	2	-	-	-	-	2	3	2
Dublin (3)	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
Newport (2)	-	-	-	-	2	1	2	1	-	2	-	-	2	2	-
London (1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total (63)	**20**	**0**	**0**	**0**	**19**	**9**	**18**	**14**	**5**	**11**	**0**	**2**	**29**	**25**	**18**
% Resistance	31.7	0	0	0	30.2	14.3	28.6	22.2	7.9	17.5	0	3.2	46.0	39.7	28.6
% Intermediate	3.2	4.8	0	0	6.3	0	0	4.8	12.7	6.3	3.2	4.8	6.3	0	0
% Susceptible	65.1	95.2	100	100	63.5	85.7	71.4	73.0	79.4	76.2	96.8	92.0	47.6	60.3	71.4

Abbreviation: Ampicillin (A); Amoxicillin/clavulanic acid (Ac); Chloramphenicol (C); Ceftazidime (Cf); Ciprofloxacin (Ci); Colistin (Co); Gentamicin (G); Kanamicin (K); Nalixidic acid (Na); Neomycin (Ne); Norfloxacin (No); Sulphonamides (Su); Streptomicin (S); Tetracycline (T); Trimethoprim (Tp); AR: Antimicrobial resistance; - Not found

Table 8. Multidrug resistance of *Salmonella* serovars isolated from retail beef at retail markets

No	MDR patterns	No. showing AR	Serovars (no.)	No	MDR patterns	No. showing AR	Serovars (no.)
1	A	1	Anatum (1)	**15**	ACSuTGK	6	Anatum (1)
2	T	1	Dublin (1), Rissen (3)	**16**	ASSuTKTp	6	Anatum (1), Typhimurium (1)
3	TTp	2	Anatum (1), Rissen (2)	**17**	CSSuTKNa	6	Newport (1)
4	ASuT	3	Rissen (3)	**18**	SSuTGKNa	6	Newport (1)
5	SuTTp	3	Anatum (1)	**19**	SSuTGKNa	6	Rissen (1)
6	ASSu	3	Derby (1)	**20**	SSuTKNeNa	6	Anatum (1)
7	ACSK	4	Derby (1)	**21**	ASTKNeTpNa	7	Rissen (1)
8	ASKTp	4	Rissen (1)	**22**	CSSuTKNeNa	7	Anatum (1)
9	ASSuT	4	Weltevreden (1)	**23**	ACSSuTGKNa	8	Typhimurium (1)
10	CSuTTp	4	Anatum (1), Derby (1)	**24**	ACSuTGKTpNa	8	Typhimurium (1)
11	SSuTTp	4	Derby (1)	**25**	ASSuTKNeTpNa	8	Anatum (1)
12	ACSGK	5	Anatum (1)	**26**	ACSSuTGTpNaCi	9	Typhimurium (1)
13	ACSuKTp	5	Anatum (2)	**27**	ACSSuTGKTpNaCi	10	Typhimurium (1)
14	CSSuGTp	5	Anatum (1)				

Abbreviation: Ampicillin (A); Amoxicillin/clavulanic acid (Ac); Chloramphenicol (C); Ceftazidime (Cf); Ciprofloxacin (Ci); Colistin (Co); Gentamicin (G); Kanamicin (K); Nalixidic acid (Na); Neomycin (Ne); Norfloxacin (No); Sulphonamides (Su); Streptomicin (S); Tetracycline (T); Trimethoprim (Tp); AR: Antimicrobial resistance

Table 9. The distribution and prevalence of resistance genes among the *Salmonella* serovars isolated from beef at retail markets

AR genes (number of isolates tested)		No. of positive isolates (%)	AR genes belonging to serovars (number positive isolates)
Ampicillin (20)			
	bla_{TEM}	18 (90.0)	Anatum (6), Derby (1), Rissen (5), Typhimurium (5), Weltevreden (1)
	bla_{OXA-1}	1 (5.0)	Typhimurium (1)
	bla_{TEM} + bla_{OXA-1}	1 (5.0)	Typhimurium (1)
	At least one gene	18 (90.0)	
Chloramphenicol (14)			
	cmlA1	7 (50.0)	Anatum (3), Typhimurium (4)
	floR	8 (57.1)	Anatum (3), Derby (2), Newport (1), Typhimurium (2)
	cmlA1+floR	2 (14.3)	Typhimurium (2)
	At least one gene	14 (100)	
Gentamicin (9)			
	aac(3)-IV	8 (88.9)	Anatum (3), Newport (1), Typhimurium (4)
Kanamycin (18)			
	aphA1-1AB	18 (100)	Anatum (8), Derby (1), Newport (2), Rissen (3), Typhimurium (4)
Streptomycin (19)			
	aadA1	15 (78.9)	Anatum (5), Derby (1), Newport (1), Rissen (3), Typhimurium (4), Weltevreden (1)
	aadA2	1 (5.3)	Typhimurium (1)
	aadA1+aadA2	1 (5.3)	Typhimurium (1)
	At least one gene	15 (78.9)	
Sulphonamides (25)			
	sul 1	20 (80.0)	Anatum (7), Derby (2), Newport (1), Rissen (4), Typhimurium (5), Weltevreden (1)
Tetracycline (29)			
	tetA	16 (55.2)	Anatum (5), Derby (1), Newport (1), Rissen (7), Typhimurium (1), Weltevreden (1)
	tetB	1 (3.4)	Derby (1)
	tetG	1 (3.4)	Dublin (1)
	At least one gene	18 (62.1)	
Trimethoprim (18)			
	dfrA1	10 (55.6)	Anatum (5), Derby (1), Rissen (4)
	dfrA12	6 (33.3)	Anatum (2), Typhimurium (4)
	At least one gene	16 (88.9)	

Table 10. Characteristic of quinolone resistance in *Salmonella* serovars isolated from beef at retail markets

Isolation (ID)	Serovars	Diameter zones of resistance (mm)			QRDRs
		Na (30 μg)	Ci (5 μg)	No (10 μg)	gyrA
HNB30	Anatum	9	22	22	Ser83Phe
HNB65	Anatum	8	21	23	Ser83Phe
HNB 21	Anatum	10	22	20	wt
HNB88	Newport	11	24	26	wt
HNB14	Newport	10	29	25	wt
HNB81	Rissen	12	30	27	wt
HNB58	Rissen	11	30	25	wt
HNB39	Typhimurium	8	17	21	Ser83Phe
HNB33	Typhimurium	8	17	21	Ser83Phe
HNB10	Typhimurium	8	14	15	Ser83Phe
HNB112	Typhimurium	6	11	13	Ser83Phe Asp87Asn

wf: Wild type

Table 11. Distribution of *Salmonella* serovars isolated from meat shops at the north Vietnam

Serovars	Number (%) of each serovar isolated from meat shops in			
	Carcass (116 samples)	Table surface (84 samples)	Sewage (45 samples)	Total (245 samples)
Infantis	16	11	5	32 (33.0)
Anatum	8	5	2	15 (15.5)
Rissen	4	5	3	12 (12.4)
Reading	6	3	2	11 (11.3)
London	4	1	1	6 (6.2)
Agona	3	1	-	4 (4.1)
Emek	3	1	-	4 (4.1)
Typhimurium	2	1	1	4 (4.1)
Newport	1	1	1	3 (3.1)
Derby	2	1	-	3 (3.1)
Enteritidis	2	-	1	3 (3.1)
Total	51	30	16	97 (100)

Table 12. Complete and intermediate resistance in *Salmonella* spp. isolated from meat shops

Antimicrobials	Carcass (51 isolates)		Table surface (30 isolates)		Sewage (16 isolates)		Total (97 isolates)	
	I no (%)	R no (%)	I no (%)	R no (%)	I no (%)	R no (%)	I no (%)	R no (%)
A	4 (7.8)	26 (51.0)	0 (0.0)	14 (46.7)	0 (0.0)	7 (43.8)	4 (4.1)	47 (48.5)
Ac	7 (13.7)	3 (5.9)	2 (6.7)	1 (3.3)	1 (6.3)	0 (0.0)	10 (10.3)	4 (4.1)
Cf	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
C	3 (5.9)	21 (41.2)	2 (6.7)	15 (50.0)	1 (6.3)	5 (31.3)	6 (6.2)	41 (42.3)
Ci	5 (9.8)	4 (7.8)	4 (13.3)	2 (6.7)	2 (12.5)	0 (0.0)	11 (11.3)	6 (6.2)
G	2 (3.9)	17 (33.3)	0 (0.0)	7 (23.3)	0 (0.0)	4 (25.0)	2 (2.1)	28 (28.9)
K	4 (7.8)	20 (39.2)	0 (0.0)	14 (46.7)	0 (0.0)	3 (18.8)	4 (4.1)	37 (38.1)
Na	4 (7.8)	21 (41.2)	1 (3.3)	13 (43.3)	1 (6.3)	5 (31.3)	6 (6.2)	39 (40.2)
Ne	3 (5.9)	16 (31.4)	3 (10.0)	10 (33.3)	2 (12.5)	3 (18.8)	8 (8.2)	29 (29.9)
No	5 (9.8)	0 (0.0)	3 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	8 (8.2)	0 (0.0)
S	1 (2.0)	23 (45.1)	1 (3.3)	14 (46.7)	0 (0.0)	6 (37.5)	2 (2.1)	43 (44.3)
Su	3 (5.9)	31 (60.8)	0 (0.0)	19 (63.3)	0 (0.0)	5 (31.3)	3 (3.1)	55 (56.7)
T	3 (5.9)	30 (58.8)	0 (0.0)	13 (43.3)	0 (0.0)	4 (25.0)	3 (3.1)	47 (48.5)
Tp	2 (3.9)	24 (47.1)	1 (3.3)	15 (50.0)	0 (0.0)	3 (18.8)	3 (3.1)	42 (43.3)

Abbreviation: Ampicillin (A), Amoxicillin/clavulanic acid (Ac), Ceftazidime (Cf), Chloramphenicol (C), Ciprofloxacin (Ci), Gentamicin (G), Kanamycin (K), Nalidixic acid (Na), Neomycin (Ne), Norfloxacin (No), Streptomycin (S), Sulphonamides (Su), Tetracycline (T), Trimethoprim (Tp); R: Resistance, I: Intermediate

Table 13. The antimicrobial resistance phenotype of *Salmonella* serovars isolated from meat shops

Number of resistance patterns	Serovars	Resistance patterns	Number of resistant antimicrobials
1	Anatum (1), Emek (1), Infantis (2)	Su	1
2	Anatum (1), Infantis (1)	T	1
3	London (1), Reading (2)	AT	2
4	Enteritidis (2)	ASSu	3
5	Newport (1)	ACTG	4
6	Rissen (1)	CTTpNa	4
7	Rissen (2)	ACSuTTp	5
8	Derby (1)	ASuTGNa	5
9	Anatum (1)	ASuTKNe	5
10	Enteritidis (1), Infantis (1), Rissen (1)	ASuTKTp	5
11	Rissen (1)	ATGKNe	5
12	Infantis (1)	CSKNeNa	5
13	Infantis (2)	ASSuKNeTp	6
14	Infantis (1), Rissen (3)	CSSuTTpNa	6
15	Rissen (2)	SuTKNeTpNa	6
16	Anatum (2)	ACSSuGNeNa	7
17	Infantis (1)	ACSSuTGAc	7
18	Infantis (2)	ACSSuTTpNa	7
19	Infantis (1)	ASSuTKNeNa	7
20	Derby (1), Infantis (1)	CSSuKNeTpNa	7
21	Rissen (1)	ACSSuGKTpNa	8
22	Anatum (1), Emek (1)	ACSSuTGKNa	8
23	Anatum (1)	ACSuTGKNeTp	8
24	Infantis (1)	ASSuTKNeTpNa	8
25	Infantis (1)	CSSuTGKNeTp	8
26	Anatum (2)	ACSSuGKNeTpNa	9
27	Infantis (1)	ACSSuGKTpNaAc	9
28	Infantis (1)	ACSSuTGKNeTp	9
29	Agona (2), Reading (2), Typhimurium (1)	ACSSuTGKTpNa	9
30	Derby (1), Typhimurium (1)	ACSSuTGTpNaAc	9
31	Infantis (1), London (1), Typhimurium (1)	ACSSuTKNeTpNa	9
32	Emek (1), Infantis (4), Typhimurium (1)	ACSSuTGKNeTpCiNa	11

Abbreviation: Ampicillin (A), Amoxicillin/clavulanic acid (Ac), Ceftazidime (Cf), Chloramphenicol (C), Ciprofloxacin (Ci), Gentamicin (G), Kanamycin (K), Nalidixic acid (Na), Neomycin (Ne), Norfloxacin (No), Streptomycin (S), Sulphonamides (Su), Tetracycline (T), Trimethoprim (Tp)

Table 14. The distribution and prevalence of resistance genes among the *Salmonella* serovars isolated from meat shops

Antimicrobial resistance genes (Number of isolates tested)	**Number of positive isolates (%)**	**Antimicrobial resistance genes belong to serovars** (Number of positive isolates)
Ampicillin (51)		
bla$_{TEM}$	38 (74.5)	Agona (2); Anatum (7); Derby (1), Emek (1); Enteritidis (1); Infantis (12); London (2); Newport (1); Reading (4); Rissen (4); Typhimurium (3)
bla$_{OXA-1}$	8 (15.7)	Emek (1); Enteritidis (2); Infantis (3); Reading (2)
bla$_{TEM}$+*bla*$_{OXA-1}$	2 (3.9)	Reading (2)
at least one gene	44 (86.3)	
Chloramphenicol (47)		
cmlA1	29 (61.7)	Agona (2); Anatum (5); Derby (2); Emek (1); Infantis (11); Newport (1); Reading (2); Rissen (2); Typhimurium (3)
floR	36 (76.6)	Agona (2); Anatum (6); Derby (1); Emek (2); Infantis (11), London (1), Newport (1), Reading (2), Rissen (6); Typhimurium (4)
cmlA1+floR	24 (51.1)	Agona (2); Anatum (5); Derby (1); Emek (1); Infantis (8); Newport (1); Reading (2); Rissen (1); Typhimurium (3)
at least one gene	41 (87.2)	
Gentamycin (30)		
aac(3)-IV	27 (90.0)	Agona (2); Anatum (5); Derby (2); Emek (1); Infantis (9), Newport (1); Reading (2); Rissen (2); Typhimurium (3)
Kanamicin (41)		
aphA1-1AB	39 (95.1)	Agona (2); Anatum (7); Derby (1); Infantis (15); London (1); Reading (4); Rissen (5); Typhimurium (4)
Steptomycin (45)		
aadA1	44 (97.8)	Agona (2); Anatum (6); Derby (2); Emek (2); Enteritidis (2); Infantis (18); London (1); Reading (2); Rissen (5); Typhimurium (4)
Suphonamides (58)		
sul 1	52 (89.7)	Agona (2); Anatum (7); Derby (3); Emek (3); Enteritidis (3); Infantis (20); Reading (2); Rissen (9); Typhimurium (4)
Tetracycline (50)		
tetA	37 (74.0)	Agona (2); Anatum (3); Derby (2); Emek (2); Enteritidis (1); Infantis (11); London (1); Reading (4); Rissen (7); Typhimurium (4)
tetB	3 (6.0)	Infantis (2); Rissen (1)
tetG	13 (26.0)	Anatum (1) ; Infantis (3) ; London (1) ; Newport (1) ; Rissen (4) ; Typhimurium (3)
tetA+tetG	8 (16.0)	Infantis (2); Rissen (3); Typhimurium (3)
at least one gene	45 (90.0)	
Trimethoprim (45)		
dfrA1	39 (86.7)	Agona (2); Anatum (3); Derby (1); Emek (1); Enteritidis (1); Infantis (16); London (1); Reading (2); Rissen (8); Typhimurium (4)
dfrA12	9 (20.0)	Derby (1); Emek (1); Infantis (6); Rissen (1)
dfrA1+dfrA12	7 (15.6)	Emek (1); Infantis (6)
at least one gene	41 (91.1)	

Appendix 1. List of the 118 *Salmonella* serovars isolated from 283 meat shops at 45 retail markets at three provinces in the north Vietnam from July 2008 to June 2009

Antibiotics	Infantis (22 isolates) no (%)	Anantum (21 isolates) no (%)	Risen (16 isolates) no (%)	Reading (13 isolates) no (%)	Emek (9 isolates) no (%)	Typhimurium (9 isolates) no (%)	London (7 isolates) no (%)	Blockey (7 isolates) no (%)	Newport (6 isolates) no (%)	Others (8 isolates) no (%)	Total (118 isolates) no (%)
A	9 (40.9)	9 (42.9)	7 (43.8)	1 (7.7)	1 (11.1)	6 (66.7)	1 (14.3)	-	3 (50.0)	2 (25.0)	39 (33.1)
Ac	-	-	-	-	-	4 (44.4)	-	-	-	-	4 (3.4)
C	7 (31.8)	8 (38.1)	6 (37.5)	3 (23.1)	4 (44.4)	5 (55.6)	2 (28.6)	-	4 (66.7)	3 (37.5)	42 (35.6)
Cf	-	-	-	-	-	-	-	-	-	-	-
Ci	-	1 (4.8)	-	-	-	5 (55.6)	-	-	-	1 (12.5)	7 (5.9)
G	2 (9.1)	4 (19.0)	4 (25.0)	-	-	5 (55.6)	-	-	2 (33.3)	1 (12.5)	18 (15.3)
K	10 (45.5)	5 (23.8)	5 (31.3)	-	-	6 (66.7)	-	-	4 (66.7)	1 (12.5)	31 (26.3)
Na	4 (18.2)	6 (28.6)	3 (18.8)	-	-	6 (66.7)	-	-	3 (50.0)	1 (12.5)	23 (19.5)
Ne	1 (4.5)	3 (14.3)	3 (18.8)	-	-	6 (66.7)	-	-	3 (50.0)	1 (12.5)	17 (14.4)
No	-	-	-	-	-	1 (11.1)	-	-	-	-	1 (0.8)
Su	18 (81.8)	7 (33.3)	11 (68.8)	3 (23.1)	5 (55.6)	6 (66.7)	2 (28.6)	1 (14.3)	4 (66.7)	5 (62.5)	62 (52.5)
S	17 (77.3)	7 (33.3)	8 (50.0)	2 (15.4)	1 (11.1)	6 (66.7)	1 (14.3)	1 (14.3)	3 (50.0)	3 (37.5)	49 (41.5)
Te	14 (63.6)	12 (57.1)	10 (62.5)	6 (46.2)	2 (22.2)	6 (66.7)	3 (42.9)	2 (28.6)	6 (100)	3 (37.5)	64 (54.2)
Tp	12 (54.5)	6 (28.6)	5 (31.3)	2 (15.4)	4 (44.4)	5 (55.6)	1 (14.3)	-	5 (83.3)	3 (37.5)	43 (36.4)

Abbreviation: Ampicillin (A), Amoxicillin/clavulanic acid (Ac), Ceftazidime (Cf), Chloramphenicol (C), Ciprofloxacin (Ci), Gentamicin (G), Kanamycin (K), Nalidixic acid (Na), Neomycin (Ne), Norfloxacin (No), Streptomycin (S), Tetracyclin (T), Trimethoprim (Tp), Sulphonamides (Su); -: not found
Other serovars including Derby (4), Weltevreden (2), Anbany (1) and Hadar (1)

Appendix 2. Pairs of primers to detect the genes encode for antimicrobial resistance

Gene/ Region		Primer pair (Forward/Reverse)	Ta/te	PCR Product	Reference
sul 1	F	CTTCGATGAGAGCCGGCGGC	65^{0}C / 00:30 min	436 bp	Guerra *et al.*,2004
	R	GCAAGGCGGAAACCCGCGCC			
aadA1a	F	GTGGATGGCGGCCTGAAGCC	70^{0}C / 00:30 min	526 bp	Guerra *et al.*,2004
	R	ATTGCCCAGTCGGCAGCG			
aadA2	F	TGTTGGTTACTGTGGCCGTA	70^{0}C / 00:30 min	381 bp	Ng et al., 1999
	R	GCTGCGAGTTCCATAGCTTC			
dfrA1	F	GTGAAACTATCACTAATGG	55^{0}C / 00:30 min	437 bp	Guerra *et al.*,2004
	R	CCCTTTTGCCAGATTTGG			
dfrA12	F	ACTCGGAATCAGTACGCA	55^{0}C / 00:30 min	463 bp	Guerra *et al.*,2004
	R	GTGTACGGAATTACAGCT			
bla_{TEM}	F	TTGGGTGCACGAGTGGGT	55^{0}C / 00:30 min	503 bp	Guerra *et al.*,2004
	R	TAATTGTTGCCGGGAAGC			
bla_{OXA-1}	F	AGCAGCGCCAGTGCATCA	60^{0}C / 00:30 min	708 bp	Guerra *et al.*,2004
	R	ATTCGACCCCAAGTTTCC			
bla_{PSE-1}	F	CGCTTCCCGTTAACAAGTAC	60^{0}C / 00:30 min	419 bp	Guerra *et al.*,2004
	R	CTGGTTCATTTCAGATAGCG			
aac(3)-IV	F	GTTACACCGGACCTTGGA	60^{0}C / 00:30 min	674 bp	Guerra *et al.*,2004
	R	AACGGCATTGAGCGTCAG			
Kn	F	ACTGGCTGCTATTGGCGA	55^{0}C / 00:30 min	525 bp	Frana et al., 2001
	R	CGTCAAGAAGGCGATAGAAGG			
aphA1-1AB	F	AAACGTCTTGCTTTCGAGGC	60^{0}C / 00:30 min	~500 bp	Guerra *et al.*,2004
	R	CAAACCGTTATTCATTCGTGA			
catA1	F	CCTGCCACTCATCGCAGT	60^{0}C / 00:30 min	623 bp	Guerra *et al.*,2004
	R	CCACCGTTGATATATCCC			
cm1A1	F	TGTCATTTACGGCATACTCG	55^{0}C / 00:30 min	435 bp	Guerra *et al.*,2004
	R	ATCAGGCATCCCATTCCCAT			
flo R	F	CACGTTGAGCCTCTATAT	55^{0}C / 00:30 min	868 bp	Guerra *et al.*,2004
	R	ATGCAGAAGTAGAACGCG			
tet A	F	GCTACATCCTGCTTGCCT	60^{0}C / 00:30 min	210 bp	Guerra *et al.*,2004
	R	CATAGATCGCCGTGAAGA			
tet B	F	TTGGTTAGGGGCAAGTTTTG	60^{0}C / 00:30 min	600 bp	Guerra *et al.*,2004
	R	GTAATGGGCCAATAACACCG			
tet G	F	GCTCGGTGGTATCTCTGC	60^{0}C / 00:30 min	500 bp	Guerra *et al.*,2004
	R	AGCAACAGAATCGGGAAC			
gyrA	F	AAATCTGCCCGTGTCGTTGGT	60^{0}C / 00:30 min	344 bp	Fabrega et al.,2009
	R	GCCATACCTACGGCGATACC			
qnrA	F	ATTTCTCACGCCAGGATTTG	55^{0}C / 00:30 min	516 bp	Gay et al., 2006
	R	GATCGGCAAAGGTTAGGTCA			
qnrB	F	GATCGTGAAAGCCAGAAAGG	55^{0}C / 00:30 min	469 bp	Gay et al., 2006
	R	ACGATGCCTGGTAGTTGTCC			
qnrS	F	ACGACATTCGTCAACTGCAA	55^{0}C / 00:30 min	417 bp	Gay et al., 2006
	R	TAAATTGGCACCCTGTAGGC			
qepA	F	CGTGTTGCTGGAGTTCTTC	59^{0}C / 00:30 min	403 bp	Cattoir et al., 2008
	R	CTGCAGGTACTGCGTCATG			
aac(6')-Ib-cr	F	ATG ACT GAG CAT GAC CTT GC	55^{0}C / 00:30 min	519 bp	Karisik et al., 2006
	R	TTA GGC ATC ACT GCG TGT TC			

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Curriculum vitae

Truong Ha Thai (Thai T.H) was borne on the 24th of July 1978 in Bac Ninh, Vietnam

1984 - 1989: received primary education at Viet Doan primary school, Viet Doan, Tien Du, Bac Ninh, Vietnam

1989 - 1993: received secondary education at the Viet Doan secondary school, Viet Doan, Tien Du, Bac Ninh, Vietnam

1993 - 1996: studied at Tien Du high school, Viet Doan, Tien Du, Bac Ninh, Vietnam.

1996 - 2001: Undergraduate student, College of Veterinary Medicine- Hanoi University of Agriculture, Vietnam

May 2002 -: work as lecturer and researcher at Department of Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, Hanoi University of Agriculture, Vietnam. Teaching two subjects: "Veterinary Epidemiology" and "Infectious diseases of domestic animal" for undergraduate students

Oct. 2004 - Dec. 2004: participated in the training program of veterinary microbiology at University de Liege, the Kingdom of Belgium.

Jul. 2007 - Mar. 2008: Trained at the Department of Pathology, Faculty of Agriculture, University of Miyazaki, Japan.

Nov. 2008 -: performed PhD research on "Antimicrobial resistance of *Salmonella* serovars isolated from meat shops at the north Vietnam" at the Department of Pathology, Faculty of Agriculture, University of Miyazaki, Japan and Department of Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, Hanoi University of Agriculture, Vietnam.

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