

Antimicrobial Susceptibilities of *Salmonella* from Domestic Animals, Food and Human in the Mekong Delta, Vietnam

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ABSTRACT. A total of 230 *Salmonella* isolates representing 33 serotypes originated from food (pork, beef, chicken meat, duck meat, and shrimp), domestic animals (pig, chicken, and duck), and human (children with diarrhea) in the Mekong Delta, Vietnam were examined for the antimicrobial resistance to 10 antibiotics. Of the 230 *Salmonella* isolates examined, 49 (21.3%) showed antimicrobial resistance. Thirty-eight isolates (16.5%) were resistant to oxytetracycline, 26 (11.3%) to chloramphenicol, 17 (7.4%) to nalidixic acid, 16 (7.0%) to streptomycin, 5 (2.2%) to kanamycin, and 4 (1.7%) to ampicillin. No isolate showed resistance to gentamicin, cefazolin, ceftriaxone, and ciprofloxacin. Among the resistant isolates, nineteen isolates were resistant to one antimicrobial agent, 10 to two, 15 to three, 3 to four, and 2 to five antimicrobial agents. The resistance rate of *Salmonella* isolates from the Mekong Delta, Vietnam to these antimicrobial agents seems to be relatively lower than the results of developed countries and even those of the neighboring countries.

KEY WORDS: antimicrobial susceptibilities, *Salmonella*, Vietnam.

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Salmonella is one of the most common pathogens causing enteritis, and it has been a major cause of foodborne disease in many countries. In recent years, there have been significant increases in the occurrence of antimicrobial resistance in *Salmonella* in both developed and developing countries [26, 28]. In developed countries, antimicrobial resistant *Salmonella* results from the use of antimicrobial agents in food animals, and these antimicrobial resistant *Salmonella* are subsequently transmitted to humans, usually through the food supply [1]. On the other hand, increase of resistant *Salmonella* in developing countries has been associated with inappropriate use of antimicrobial agents in human medicine [26].

Because of public health concerns, antimicrobial resistance surveillance networks have been created in veterinary and human medicine [1, 9, 18, 19, 35]. Thus, a lot of information about prevalence and antimicrobial resistance of *Salmonella* are available in developed countries, notably in North America and Europe. Nevertheless, few data, especially of non-human *Salmonella* isolates, are available in developing countries. In Vietnam, the few reports dealing with antimicrobial resistance of *Salmonella* isolates are of human isolates [14, 24], and there are a few information about prevalence and antimicrobial resistance of *Salmonella* of other sources [32,33]. However, Heinitz *et al.* [12] reported that 30% of *Salmonella* isolates originated from imported seafoods from Vietnam to United States from

1990 to 1998 were resistant to some antimicrobial agents. Kiessling *et al.* [15] also reported the prevalence of antimicrobial resistant *Salmonella* in food samples imported from Vietnam to United States from 1999 to 2000, and among those isolates, one *Salmonella* Derby isolated from frozen eel showed resistance to seven antimicrobial agents. Thus, it is important to know the prevalence of resistant *Salmonella* in Vietnam, not only for public health in Vietnam, but also in view of food exporting country. The Mekong Delta consisting of 12 provinces and 1 city is located in the southern area of Vietnam, and 3 millions pigs and 44 million poultry were raised in this area in 2000. However, no reports have been published regarding the antimicrobial susceptibility of *Salmonella* spp. originated from the Mekong Delta. Therefore, in this study, the antimicrobial susceptibility of *Salmonella* originated from various sources in the Mekong Delta in Vietnam was examined.

MATERIALS AND METHODS

Bacterial isolates: A total of 230 *Salmonella* isolates representing 33 serotypes originated from food (pork, beef, chicken meat, duck meat, and shrimp), domestic animals (pig, chicken, and duck), and human (children with diarrhea) were examined (Table 1). These were isolated from July 1999 to September 2001 in 8 provinces in the Mekong Delta, Vietnam [29,30]. Food (pork, beef, chicken meat, duck meat, and shrimp) were originated from wet markets in the Mekong Delta. Sample of domestic animals (pig, chicken, and duck) were originated from farm. Human samples were originated from the patient which visited hospitals in the Mekong Delta, Vietnam.

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Table 1. Serotype distribution of 230 *Salmonella* isolates from Vietnam

Serotypes	Food					Animal			Human	Total
	Pork	Beef	Chicken meat	Duck meat	Shrimp	Pig	Chicken	Duck		
<i>S. Aberdeen</i>						1				1
<i>S. Anatum</i>	5		2	1			1			9
<i>S. Blockley</i>			1	1						2
<i>S. Bovismorbificans</i>	1	1		1	1			2	2	8
<i>S. Braenderup</i>								1		1
<i>S. Derby</i>	11	1		1	3	4		1		21
<i>S. Dessau</i>	2	5	2	2	4					15
<i>S. Dublin</i>				2				1		3
<i>S. Emek</i>			2				8			10
<i>S. Enteritidis</i>							1			1
<i>S. Hadar</i>			2	1						3
<i>S. Javiana</i>			1			8	4	3		16
<i>S. Lexington</i>	2		2	2	1			1		8
<i>S. Lome</i>								1		1
<i>S. London</i>	4	6	1	2		1			1	15
<i>S. Mbandaka</i>	1									1
<i>S. Newport</i>	2		1					1		4
<i>S. Norwich</i>	1									1
<i>S. Ohio</i>									3	3
<i>S. Schleissheim</i>	1				1					2
<i>S. Senftenberg</i>								2		2
<i>S. Singapore</i>							1			1
<i>S. Southampton</i>							1			1
<i>S. Stanley</i>	1			1		1				3
<i>S. Tennessee</i>	2		1		7	1				11
<i>S. Thompson</i>					1					1
<i>S. Typhimurium</i>			2	1		3	1	6	1	14
<i>S. Virchow</i>					1	1				2
<i>S. Wagania</i>								1		1
<i>S. Weltevreden</i>	13	17		1	8	3	3	4		49
<i>S. Westhampton</i>	2									2
<i>S. Worthington</i>					1					1
<i>S. II heilbron</i>						1				1
UT ^{a)}		5	3	4	1	1		1	1	16
Total	48	35	20	20	29	26	19	25	8	230

a) Untypable.

Antimicrobial susceptibility test: *Salmonella* isolates were examined for susceptibility to 10 different antimicrobial agents by agar dilution method according to the National Committee for Clinical Laboratory Standards (NCCLS) procedure M7-A5 [21]. The antimicrobial agents used were ampicillin (ABPC), streptomycin (SM), kanamycin (KM), gentamicin (GM), oxytetracycline (OTC), chloramphenicol (CP), cefazolin (CEZ), ceftriaxone (CTRX), nalidixic acid (NA), and ciprofloxacin (CPFX). Antimicrobial susceptibility was assessed following the NCCLS procedure, but isolates showing intermediate susceptibility were classified as susceptible. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 were used as a control strain according to the NCCLS.

Statistical analysis: The Chi-square test and Fisher's

exact test were used for statistical analysis of the significant difference of resistant rates.

RESULTS

Of the 230 *Salmonella* isolates examined, 49 (21.3%) showed antimicrobial resistance. Thirty-eight isolates (16.5%) were resistant to oxytetracycline, 26 (11.3%) to chloramphenicol, 17 (7.4%) to nalidixic acid, 16 (7.0%) to streptomycin, 5 (2.2%) to kanamycin, and 4 (1.7%) to ampicillin. None of the isolates showed resistance to gentamicin, cefazolin, ceftriaxone, and ciprofloxacin (Table 2).

Of the 123 isolates from retail meat, a total of 33 (26.8%) isolates composed of 18 (37.5%) isolates from pork, 2 (5.7%) from beef, 9 (45%) from chicken meat, and 4

Table 2. Antimicrobial resistance of *Salmonella* isolates by food, domestic animals, and humans in Vietnam

Source	No. of examined	No. of resistant isolates ^{a)} (%)										No. of Resistance ^{b)} (%)
		ABPC	SM	KM	GM	OTC	CP	CEZ	CTRX	NA	CPFX	
Food												
Pork	48	3 (6.3)	7(14.6)			10 (20.8)	6 (12.5)					18 (37.5) ^{c)}
Beef	35		2 (5.7)			1 (2.9)	1 (2.9)					2 (5.7) ^{c,d)}
Chicken meat	20	1 (5.0)	4(20.0)	4(20.0)		9 (45.0)	5 (25.0)			7 (35.0)		9 (45.0) ^{d)}
Duck meat	20		3(15.0)	1 (5.0)		3 (15.0)	1 (5.0)			1 (5.0)		4 (20.0)
Shrimp	29					2 (6.9)	1 (3.4)					2 (6.9)
Animal												
Chicken	19					8 (42.1)	8 (42.1)			9 (47.4)		9 (47.4) ^{e,f)}
Duck	25					1 (4.0)	1 (4.0)					1 (4.0) ^{e)}
Pig	26					3 (11.5)	3 (11.5)					3 (11.5) ^{f)}
Human	8					1 (12.5)						1 (12.5)
Total	230	4 (1.7)	16 (7.0)	5 (2.2)	0	38 (16.5)	26 (11.3)	0	0	17 (7.4)	0	49 (21.3)
MIC ₅₀ (μg/ml)												
		1	8	2	0.5	2	8	1	<0.125	4	<0.125	
MIC ₉₀ (μg/ml)												
		2	8	2	0.5	128	32	1	<0.125	32	<0.125	

a) ABPC: Ampicillin, SM: Streptomycin, KM: Kanamycin, GM: Gentamicin, OTC: Oxytetracycline, CP: Chloramphenicol, CEZ: Cefazolin, CTRX: Ceftriaxone, NA: Nalidixic acid, CPFX: Ciprofloxacin.

b) Isolates resistant to at least one antimicrobial agent.

c, d, e, f) Difference is statistically significant, $P<0.01$.

Table 3. Antimicrobial resistance patterns of *Salmonella* isolates from Vietnam

No. of antimicrobial agents	Resistance pattern ^{a)}	No. of resistant isolates	Serotypes	Source
1	SM	9	<i>S. Derby</i>	Pork (7), Beef (1), Duck meat (1)
	OTC	8	<i>S. Derby</i>	Shrimp (1)
			<i>S. Anatum</i>	Pork (2), Duck meat (1)
			<i>S. London</i>	Pork (1), Chicken meat (1), Human (1)
			<i>S. Norwich</i>	Pork (1)
	CP	1	<i>S. Derby</i>	Pork (1)
	NA	1	<i>S. Enteritidis</i>	Chicken (1)
	ABPC+OTC	1	<i>S. Anatum</i>	Pork (1)
	SM+OTC	1	<i>S. Hadar</i>	Duck meat (1)
	OTC+CP	8	<i>S. Derby</i>	Pork (3), Shrimp (1), Pig (3), Duck (1)
3	ABPC+OTC+CP	3	<i>S. Anatum</i>	Pork (2)
			<i>S. Javiana</i>	Chicken meat (1)
	SM+OTC+CP	1	<i>S. Weltevreden</i>	Beef (1)
	OTC+CP+NA	11	<i>S. Hadar</i>	Chicken meat (1)
4			<i>S. Emek</i>	Chicken meat (2), Chicken (8)
	SM+KM+OTC+NA	3	<i>S. Blockley</i>	Chicken meat (1)
			UT	Chicken meat (2)
5			<i>S. Blockley</i>	Duck meat (1)
	SM+KM+OTC+CP+NA	2	<i>S. Hadar</i>	Chicken meat (1)
Total		49		

a) ABPC: Ampicillin, SM: Streptomycin, KM: Kanamycin, OTC: Oxytetracycline, CP: Chloramphenicol, NA: Nalidixic acid.

(20.0%) from duck meat, of the 70 isolates from domestic animals, a total of 13 (18.6%) isolates composed by 9 (47.4%) from chicken, 1 (4.0%) from duck, and 3 (11.5%) from pig, and of the 29 isolates from retail shrimp, two (6.9%) isolates, and of the 8 isolates from human, 1 (12.5%) isolate showed resistance to some antimicrobial agents. The resistance rates of *Salmonella* isolates from pork and chicken meat were significantly higher than that from beef

($P<0.01$), and among the isolates from domestic animals, resistant rates from chicken were significantly higher than those from ducks and pigs ($P<0.01$) (Table 2).

Among the resistant isolates, nineteen isolates showed resistance to one antimicrobial agent, 10 to two, 15 to three, 3 to four, and 2 to five antimicrobial agents (Table 3).

All of 17 NA resistant isolates were less susceptible to CPFX (MIC=0.25–2 μ g/ml) when compared to the other

isolates.

DISCUSSION

Of the 10 antimicrobial agents analyzed in this study, *Salmonella* isolated in the Mekong Delta, Vietnam showed resistance to 6 agents. In developed countries, high resistance rates have generally been observed against those antimicrobial agents used since early times. In Japan, Miwa *et al.* [20] reported that 182 (63.4%), 184 (64.8%), and 20 (7.0%) among 287 *Salmonella* isolates, originated from chicken carcasses from 1996 to 1999, were resistant to OTC, SM, and ABPC, respectively, and Takahashi *et al.* [25] reported that 100 (80.6%), 95 (76.6%), and 11 (8.9%) among 124 isolates from feces of healthy domestic animals, mainly broiler chicken, in 1999 were resistant to OTC, dehydrostreptomycin (DSM), and ABPC, respectively. White *et al.* [36] reported that in the United States, 36 (80%), 33 (73%), and 12 (27%) among 45 of *Salmonella* isolates from retail ground meat in 1998 were resistant to tetracycline (TC), SM, and ABPC, respectively; and Poppe *et al.* [22] reported that in Canada, 341 (25.5%), 354 (26.5%), and 212 (15.9%) of 1336 isolates from animals, animal food products, and the environment of animal production were resistant to the same respective antimicrobial agents as in the United States. Similarly, resistance to those antimicrobial agents has been reported in developing countries in Southeast Asia. Rasrinaul *et al.* [23] reported that in Thailand, 40 (46%), 12 (14%), and 5 (6%) of 87 *Salmonella* isolates from food samples in 1986 were resistant to TC, SM, and ABPC, respectively. Van *et al.* [32] reported that 91 *Salmonella* isolates from retail raw food samples obtained in Ho Chi Minh city, Vietnam were resistant to ABPC (22%), amoxicillin (22%), TC (40.7%), KM (2.2%), GM (2.2%), SM (14.3%), sulfafurazole (16.5%), enrofloxacin (8.8%), CP (2.2%), trimethoprim (3.3%) and NA (18.4%). The *Salmonella* isolates from the Mekong Delta, Vietnam also showed resistance to OTC, CP, NA, SM, KM, and ABPC. However, when compared with the results of developed countries and even with the results of neighboring countries, it seems that the resistance rate of *Salmonella* isolates from the Mekong Delta to these antimicrobial agents was relatively low. Furthermore, in the United States, besides resistance to those antimicrobial agents used since early times, resistance to CTRX, a third generation cephalosporin used to treat children with *Salmonella* infection, was detected in 7 (16%) of 45 *Salmonella* isolates in retail ground meat in 1998 [36]. Likewise, Boonmer *et al.* [2] reported that 28 (14.1%) of 199 *Salmonella* isolates from frozen chicken meat in Thailand were also resistant to CTRX. However, CTRX resistant *Salmonella* isolates as well as isolates resistant to relatively new antimicrobial agents were not detected in the Mekong Delta in Vietnam. Van *et al.* [32] also reported that no *Salmonella* isolates originated from retail raw food samples obtained in Ho Chi Minh city, Vietnam showed resistance to cephalothin, a third generation cephalosporin. This would be partly

because intensive livestock production, where antimicrobial agents are used as growth promoter or feed additives, is rare in Vietnam, and most of domestic animals are raised in small farm or farmer yard and fed agricultural by-products.

Moreover, in developed countries, the spread of particular serotypes and phage types that acquired multi-drug resistance has become an increasing public health problem. In the United States, the three most common *Salmonella* serotypes (Typhimurium, Enteritidis, and Newport) accounted for 50% of clinical isolates from human and 44% of clinical animals in 2001, and among them, *S. Typhimurium* and *S. Newport* have emerged as major multi-drug resistant pathogens [4]. An increase in the incidence of food-borne infections caused by *S. Enteritidis*, and human and animal infections by multi-drug resistant strains of *S. Typhimurium* has been also observed in European countries [3, 6, 31]. Among the multi-drug resistant *Salmonella*, *S. Typhimurium* definitive type DT104 has emerged as a global health problem in human and animal medicine during the last decade because of its resistance to up to nine antimicrobial agents commonly used [8, 13, 26, 27]. Van *et al.* [32] reported that 20.9% of 91 *Salmonella* isolates from retail raw food samples showed multi-drug resistance. In the Mekong Delta, many of the above serotypes have also been isolated, including *S. Typhimurium*, but no resistant strain of this serotype was detected in this study. On the other hand, *S. Blockley*, *S. Hadar*, and *S. Emek* showed a higher rate of multi-drug resistance. Those multi-drug resistant isolates were mainly originated from chicken, chicken meat and duck meat. It is not clear why mainly chicken related isolates showed a tendency to multi-drug resistance, but it might be associated with recent introduction of some commercial chicken farms using feed sold by the United States or European feed companies, in which antimicrobial agents are including in the feed.

This is the first report of quinolone resistance in non-typhoidal *Salmonella* isolated in the Mekong Delta in Vietnam, although Van *et al.* reported that *Salmonella* showing the resistance against enrofloxacin were isolated from retail raw food samples in Ho Chi Minh city in 2004 [32]. In addition, all the NA resistant isolates in the present study showed reduced susceptibility to CPF. Hakanen *et al.* [11] suggested much lower MIC breakpoints values (MIC 0.25 µg/ml) for the fluoroquinolones than those recommended by the NCCLS (MIC 4 µg/ml) because of clinical importance of low-level CPF resistance. If we adopt that breakpoint for the *Salmonella* isolates from the Mekong Delta in Vietnam, 4.8% (11 per 230) of the isolates should be considered resistant to ciprofloxacin. It is generally said that resistance to NA is a first-step of resistance to fluoroquinolones. Moreover, fluoroquinolones have been considered to be efficient antimicrobial agents against *Salmonella* infections and have been widely used. Therefore, as the emergence of quinolone resistance in *Salmonella* can be a serious public health problem, introduction of fluoroquinolones as food additives in food-producing animals, which would be a cause of inducing fluoroquinolone resistance to *Salmonella*,

is a cause for particular concern [10, 17, 35, 37, 38]. The results of this study, where quinolone resistant strains were detected in *Salmonella* isolates from animals and foods in Vietnam, suggested that fluoroquinolones are used in animal production as food additives or for treatment. Indeed, fluoroquinolones, such as norfloxacin and enrofloxacin, are sold at retail pharmacy for treatment of animal salmonellosis, but the detailed relationship among use of those antimicrobial agents and acquisition of quinolone resistance in the Mekong Delta is unclear due to the absence of reliable data on antimicrobial agents supplied to animals. However, the development of quinolone resistance, like in other countries, should also be considered.

Prudent use of antimicrobials in animal production system has been agreed worldwide to prevent development of antimicrobial resistance in pathogenic bacteria [5]. However, self-medication through retail pharmacies, which is a common practice in developing countries, is recognized as one of causes of inducing antimicrobial resistance in pathogenic bacteria. Similar to other developing countries, in Vietnam, antimicrobial agents can be bought easily in pharmacies without a prescription [5, 7]. Larsson *et al.* [16] also reported that ABPC, penicillin, amoxicillin, erythromycin, TC, and SM, respectively, are the most commonly used antimicrobial agents for treatment to acute respiratory tract infection in children in Bavi, Vietnam, and among the pathogens isolated from children, 88% of *Streptococcus pneumoniae* isolates and 32% of *Haemophilus influenzae* were resistant to TC, and 18% of *H. influenzae* and 19% of *Moraxella catarrhalis* were resistant to ABPC. Considering that many antimicrobial agents for human and animals can be bought without control and therefore used inappropriately, increase of the antimicrobial resistance among *Salmonella* in Vietnam in future should be considered. Therefore, further investigation of *Salmonella* isolates from more extensive sources and continuous monitoring of antimicrobial resistance in Vietnam must be of great concern.

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