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Detection of *Salmonella* spp. in Retail Raw Food Samples from Vietnam and Characterization of Their Antibiotic Resistance▽

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A study was conducted to examine the levels of *Salmonella* spp. contamination in raw food samples, including chicken, beef, pork, and shellfish, from Vietnam and to determine their antibiotic resistance characteristics. A total of 180 samples were collected and examined for the presence of *Salmonella* spp., yielding 91 *Salmonella* isolates. Sixty-one percent of meat and 18% of shellfish samples were contaminated with *Salmonella* spp. Susceptibility of all isolates to a variety of antimicrobial agents was tested, and resistance to tetracycline, ampicillin/amoxicillin, nalidixic acid, sulfafurazole, and streptomycin was found in 40.7%, 22.0%, 18.7%, 16.5%, and 14.3% of the isolates, respectively. Resistance to enrofloxacin, trimethoprim, chloramphenicol, kanamycin, and gentamicin was also detected (8.8 to 2.2%). About half (50.5%) of the isolates were resistant to at least one antibiotic, and multiresistant *Salmonella* isolates, resistant to at least three different classes of antibiotics, were isolated from all food types. One isolate from chicken (serovar Albany) contained a variant of the *Salmonella* genomic island 1 antibiotic resistance gene cluster. The results show that antibiotic resistance in *Salmonella* spp. in raw food samples from Vietnam is significant.

Food-borne diseases are an important cause of morbidity and mortality worldwide. The worldwide incidence of nontyphoidal salmonellosis is estimated at 1.3 billion cases and 3 million deaths annually (45). Although *Salmonella* gastroenteritis is generally a self-limiting illness, severe cases may require antimicrobial therapy.

Food contamination with antibiotic-resistant bacteria can be a major threat to public health, as the antibiotic resistance determinants can be transferred to other pathogenic bacteria, potentially compromising the treatment of severe bacterial infections. The prevalence of antimicrobial resistance among food-borne pathogens has increased during recent decades (6, 14, 18, 25, 47). This increase is attributed to the selection pressure created by using antimicrobials in food-producing animals, in addition to the unregulated use of antibiotics by humans in developing countries (1, 4, 9, 46, 49).

Salmonella genomic island 1 (SGI1) is the first genomic island reported to contain an antibiotic resistance gene cluster and was identified in the multidrug-resistant *Salmonella enterica* serovar Typhimurium DT104. The antibiotic resistance genes are clustered in a 13-kb segment within a 43-kb genomic island. All resistance genes are contained within a complex integron structure containing the *aadA2* gene (streptomycin [STR] and spectinomycin resistance gene), a truncated *sul1* (*sul1*Δ) gene, the *pse-1* gene (ampicillin [AMP] resistance gene), and a complete *sul1* gene (sulfonamide resistance gene). Other resistance genes, including *floR* (chloramphenicol [CHL]/florfenicol resistance gene) and the tetracycline (TET) resistance genes *tetR* and *tetA*(G) are contained within the integron (31) (Fig. 1). Until now, SGI1 and its variants have been identified in 13 serovars in many countries, mostly from human and animal sources, but isolates carrying SGI1 or its derivatives have not been reported from Vietnam. The SGI1 variants were designated A to L (16, 32, 34).

Like in many other developing countries, raw food hygiene and antimicrobial resistance epidemiology are in their infancy in Vietnam. In addition, the lack of stringent controls on antimicrobial usage in human health and particularly in animal production systems increases the risk of food-borne microbes harboring an array of resistance genes. The objective of this study was to determine the prevalence of *Salmonella* spp. in foods commonly sold in the marketplace in Ho Chi Minh City, Vietnam. The phenotypic aspects of the isolates were then identified by antibiotic susceptibility tests against 15 antibiotics, and isolates were examined for the presence of SGI1, which is recognized as an emerging problem worldwide.

MATERIALS AND METHODS

Isolation and identification of *Salmonella* spp. One hundred eighty samples of meat, comprising beef ($n = 50$), chicken/poultry ($n = 30$), pork ($n = 50$), and shellfish ($n = 50$), were purchased from 14 markets and 4 supermarkets around Ho Chi Minh City between February and June 2004 for the isolation and identification of *Salmonella* spp. Each market was sampled only once. The procedures for isolation of *Salmonella* spp. were based on the Nordic Committee on Food Analysis method (37). The isolates were further grouped with commercial monovalent sera (Remel), which were used according to the manufacturer's instructions. Representative isolates were further serotyped by the Microbiological Diagnostic Unit, Melbourne University, Australia.

Antibiotic susceptibility tests. All 91 *Salmonella* isolates recovered from beef (32 isolates), pork (32 isolates), poultry (18 isolates), and shellfish (9 isolates) were tested for their resistance to 15 antimicrobial agents by the disk diffusion method on Mueller-Hinton agar plates. The standard procedure of the CLSI (formerly NCCLS) (35) were strictly followed throughout the testing procedure. Quality control strains *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were included in each run. The amounts of antimicrobial agents on the disks (Oxoid, Australia) were as follows: AMP, 10 μg; amoxicillin (AMX), 10

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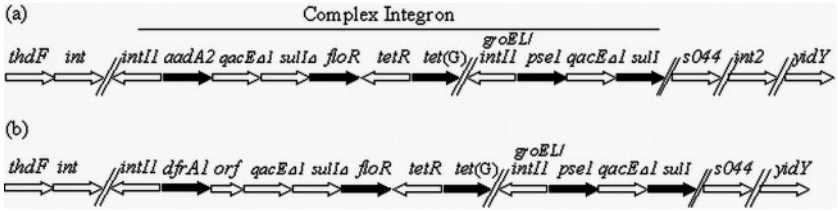

FIG. 1. Antibiotic resistance gene cluster. (a) SGI1 of *Salmonella* serovar Typhimurium DT104 (GenBank accession number AF261825). (b) SGI1-F of *Salmonella* serovar Albany. The black arrows correspond to antibiotic resistance genes. Genes are not drawn to scale.

μg; augmentin (AMC), 30 μg; cephalothin (CEF), 30 μg; CHL, 30 μg; ciprofloxacin (CIP), 5 μg; enrofloxacin (ENR), 5 μg; TET, 30 μg; gentamicin (GEN), 10 μg; kanamycin (KAN), 30 μg; nalidixic acid (NAL), 30 μg; norfloxacin (NOR), 10 μg; sulfafurazole (SUL), 300 μg; STR, 10 μg; and trimethoprim (TMP), 5 μg. The isolates were classified as susceptible, intermediate, and resistant according to the zone diameter interpretative standards recommendations by CLSI (17). The Mann-Whitney test using MINITAB was performed to ascertain significant differences in the degrees of resistance of *Salmonella* isolates between food sources.

Mapping of SGI1. Multiresistant isolates were first examined for the presence of class 1 integrons by PCR using primers and conditions as previously described (33). The integron-positive isolates were investigated for the presence of SGI1 by PCR mapping using the primers described in Table 1. Three isolates were subjected to this study: S/C/21a, S/SF/8a, and S/P/9. *Salmonella* serovar Typhimurium DT104, which harbored SGI1, was used as a positive control. Template DNA was prepared using the Wizard DNA purification kit (Promega). The isolates were first examined by PCR for the presence of the left and right junctions of SGI1. Isolates which were positive for these PCRs were further assessed for the presence of other resistance genes in SGI1 by PCRs, and all PCR amplification products were sequenced. To detect genes in the integron 1 region in the isolate S/C/21a, PCR amplification was performed with the primer pair int1-F3 (Table 1 and Fig. 1). Internal primers were then synthesized and the amplification product was sequenced, allowing identification of the resistance genes in this region. In addition, to confirm the same arrangement of resistance genes in SGI1 of the isolate S/C/21a and the positive control strain (*Salmonella* serovar Typhimurium DT104), long PCRs using primer pairs sulTER-tetA, tetR-pse1, and pseL-MDRB were performed with both the S/C/21a isolate and the positive control strain, and then PCR amplicons of the same size from positive control and tested isolate were restriction digested with at least three restriction endonucleases and were considered identical if they had the same restriction fragment length polymorphism patterns after digestions. Long-PCR amplifications were performed using Expand long-template DNA polymerase (Roche, Germany), with the PCR mixture and conditions used according to the manufacturer's instructions and an annealing temperature of 57°C. For all other primer pairs, PCRs were carried out in a total volume of 50 μl containing 100 ng of DNA template, each deoxyribonucleotide at a concentration of 0.25 mM, 2 mM $MgCl_2$, 1 U of AmpliTaq Gold DNA polymerase (Roche, Germany), and 0.4 μM of each primer. Thermal cycler reaction conditions included an initial denaturation at 95°C for 5 min, followed by 25 cycles of denaturation at 94°C for 30 s, annealing for 1.5 min (at 57°C for all primer pairs except IABF-IABR and sulTER-F3, which were at 60°C), and extension at 72°C for 3 min and final cycle of amplification at 72°C for 10 min.

RESULTS

Presence of *Salmonella* spp. in meat, poultry, and shellfish. The present study highlights the considerably high prevalence of *Salmonella* spp. in raw meat and poultry, in which 32/50 (64%) of pork samples, 31/50 (62%) of beef samples, and 16/30 (53.3%) of chicken samples were contaminated with *Salmonella* spp. However, the rate of *Salmonella* contamination in shellfish (18.0%) was much lower than that in meat and poultry (60.8%).

Antibiotic resistance of *Salmonella* isolates. Ninety-one *Salmonella* isolates recovered from food samples were tested for antibiotic resistance against 15 antibiotics. The antibiotic resistance rates for each source and for the whole set of isolates

TABLE 1. Primers used for PCR for mapping of SGI1

Primer name	Sequence (5′→3′)	Gene	Amplification	Size (bp)	Reference
Int1-F	GGCATCCAAGCAGCAAGC		Class 1 integrons	Variable	33
Int1-R	AAGCAGACTTGACCTGAT				33
U7-L12	ACACCTTGAGCAGGGCAAG	*thdF*	Left junction *thd-F* to *int*	500	20
LJ-R1	AGTTCTAAAGGTTCGTAGTCG	*int*			20
104-RJ	TGACGAGCTG F AGCGAATTG	S044	Right junction		20
C9-L2	AGCAAGTGTGCGTAATTTGG	*int2*	S044 to *int2*	515	20
104-D	ACCAGGGCAAAACTACACAG	*yidY*	S044 to *yidY*	500	20
cml01	TTTGGWCCGCTMTCRGAC	*floR*	*floR*	494	20
cml15	SGAGAARAAGACGAAGAAG	*floR*			20
int1	GCTCTCGGGTAACATCAAGG	*intI1*	*intI1* to *aadA2*	1,135	20
aad	GACCTACCAAGGCAACGCTA	*aadA2*			20
sulTER	AAGGATTTCCTGACCCTG	*sul1*Δ	*sul1* to *floR*	942	20
F3	AAAGGAGCCATCAGCAGCAG	*floR*			20
F4	TTCCTCACCTTCATCCTACC	*floR*	*floR* to *tetR*	598	20
F6	TTGGAACAGACGGCATGG	*tetR*			20
tetR	GCCGTCCCGATAAGAGAGCA	*tetR*	*tetR* to *tetA*	1,559	20
tetA	GAAGTTGCGAATGGTCTGCG	*tetA*			20
int2	TTCTGGTCTTCGTTGATGCC	*groEL-intI1*	*int1* to *pse1*	1,338	20
pse1	CATCATTTCGCTCTGCCATT	*pse-1*			20
pseL	AATGGCAATCAGCGCTTCCC	*pse-1*	*pse-1* to S044	4,400	20
MDRB	GAATCCGACAGCCAACGTTCC	*S044*			20
IABF	GGAGTGCCAAAGGTGAACAG	*dfrA1*	*dfrA1* to *sul1*	2,385	This study
IABR	CGAAGAACCGCACAATCTCG	*sul1*Δ		2,385	This study

TABLE 2. Percentages of *Salmonella* isolates resistant to antibiotics from various food sources

Antibiotic	% of resistant isolates from:				
	Pork (n = 32)	Beef (n = 32)	Chicken (n = 18)	Shellfish (n = 9)	All sources (n = 91)
AMP	50.0	0.0	22.2	0.0	22.0
AMX	50.0	0.0	22.2	0.0	22.0
AMC	0.0	0.0	0.0	0.0	0.0
TET	78.1	12.5	38.9	11.1	40.7
SUL	18.8	9.4	33.3	0.0	16.5
KAN	3.1	0.0	0.0	11.1	2.2
GEN	3.1	0.0	5.6	0.0	2.2
STR	15.6	6.3	27.8	11.1	14.3
NOR	0.0	0.0	0.0	0.0	0.0
ENR	12.5	0.0	22.2	0.0	8.8
CIP	0.0	0.0	0.0	0.0	0.0
NAL	25.0	6.3	38.9	0.0	18.7
CHL	0.0	0.0	11.1	0.0	2.2
CEF	0.0	0.0	0.0	0.0	0.0
TMP	3.1	0.0	5.6	11.1	3.3
Resistance to ≥1 antibiotic	78.1	12.5	88.9	11.1	50.5
Multiresistance	34.4	6.3	27.8	11.1	20.9

are represented in Table 2. There were 40.7% of *Salmonella* isolates resistant to TET, for which resistance was mostly observed in pork isolates, where 78.1% of the isolates were resistant to this antibiotic. Resistance to AMP/AMX, NAL, SUL, and STR was found in 22.0%, 18.7%, 16.5%, and 14.3% of the isolates, respectively. Resistance to ENR, TMP, CHL, KAN, and GEN was also detected (8.8 to 2.2%). In addition, it was observed that approximately half (50.5%) of the isolates were resistant to at least one antibiotic; the highest rates were in pork and chicken samples (78.1 and 88.9%, respectively). Multiresistant *Salmonella* isolates were observed in all food types. The rates were 34.4%, 27.8%, 11.1%, and 6.3% in pork, chicken, shellfish, and beef isolates, respectively. In general, *Salmonella* isolated from chicken and pork showed a greater degree of resistance than that from beef and shellfish (at a significance level of 0.05), reflecting the higher use of antibiotics in poultry and pig farming.

There were 18 *Salmonella* isolates showing resistance to multiple antibiotics. Resistance to TET, AMP/AMX, SUL, and NAL was the common features of these multiresistant isolates. Multiresistance was predominant in serogroup E isolates, including *Salmonella* serovars London and Anatum. In addition, there was an isolate from pork belonging to serovar Typhimurium that showed resistance to an additional four antibiotics, being resistant to GEN, SUL, TMP, STR, and KAN. A serogroup C2 isolate (serovar Albany) from chicken was resistant to seven antibiotics (Table 3).

Presence of SGI1 in *Salmonella* isolates. The *Salmonella* integron-positive isolates (S/C/21a, S/P/9, and S/SF/8a) were investigated for the presence of SGI1. Isolate S/C/21a (serovar

TABLE 3. Antibiotic resistance, serogroups, and serotypes of all multiresistant *Salmonella* isolates

Isolate	Source[a]	Resistance[b] to:										*Salmonella* serovar (serogroup)
		AMP/AMX	TET	GEN	CHL	SUL	TMP	STR	KAN	NAL	ENR	
S/C/3	C		R			R		R		R	R	London (E)
S/C/5	C	R		R	R	R						Havana (G2)
S/C/9a	C		R					R		R		Hadar (C2)
S/C/21a	C	R	R		R	R	R			R		Albany (C2)
S/C/23b	C		R			R		R				London (E)
S/B/19a	B		R			R		R		R		Anatum (E)
S/P/3	P	R	R							R	R	Anatum (E)
S/P/5a	P	R	R							R		Anatum (E)
S/P/7	P	R	R							R		Anatum (E)
S/P/9	P	R	R	R		R	R	R	R			Typhimurium (B)
S/P/13	P		R			R		R				Anatum (E)
S/P/15	P	R	R							R		Anatum (E)
S/P/16	P		R			R		R		R	R	London (E)
S/P/17	P	R	R							R	R	Anatum (E)
S/P/18	P	R	R							R	R	Anatum (E)
S/P/24	P	R	R			R		R				Anatum (E)
S/P/25b	P		R			R		R				Anatum (E)
S/SF/8a	SF		R				R		R			Typhimurium (B)

[a] C, chicken; B, beef; P, pork; SF, shellfish.
[b] R, resistant.

Albany) was positive by PCR for the left junction (*thdF* and *int* gene) and right junction without retron (S044-*yidY*). There was no amplification product obtained with the PCR primer set for the S044-*int2* fragment (right junction with retron), and therefore the int2 retron sequence was absent in this isolate. This is consistent with other reports that the retron sequence found downstream of SGI1 in *Salmonella* serovar Typhimurium is lacking in other serovars (7, 20, 21). PCR mapping of the antibiotic resistance gene cluster of isolate S/C/21a demonstrated that instead of the *aadA2* gene classically found in the first segment of the complex integron of the SGI1 antibiotic resistance gene cluster, the *dfrA1* (TMP resistance gene) and *orfC* (of unknown function) were found in the corresponding intergon of serovar Albany strain S/C/21a (Fig. 1). This SGI1 variant has previously been classified as SGI1-F (34).

DISCUSSION

The present study demonstrated that retail raw meat and poultry samples from markets and supermarkets in Ho Chi Minh City, Vietnam, were heavily contaminated with *Salmonella* spp. (60.8%). This very high level of contamination indicates a potential breakdown of hygiene at various stages of the food processing and distribution chain and/or a lack of refrigeration of meat. In addition, 18% of shellfish samples were contaminated with *Salmonella* spp. Since shellfish are often eaten raw or after minimal cooking, the results illustrate the potential for shellfish also to cause enteric disease. The result for *Salmonella* contamination in pork samples (64.0%) was in close agreement with that of Phan et al. (40), who reported that 69.9% of the retail pork samples were contaminated with *Salmonella* spp. in the Mekong Delta, Vietnam. However, we have found the levels of *Salmonella* contamination in retail beef and chicken meat samples were much higher (62.0% and 53.3%) than those reported by Phan et al. (48.6% for beef samples and 21.0% for chicken meat). The present study was conducted in Ho Chi Minh City, and the differences noted may include regional variability in the country and a longer time to market of slaughtered products. The reported rates of *Salmonella* contamination in retail meat and poultry are lower in more developed countries. In this study, 53.3% of poultry samples were contaminated with *Salmonella*, compared to only 23 to 29% in the United Kingdom (27, 41), 2.8 to 26.4% in Ireland (24, 29), 13.2% in The Netherlands (50), 35.8% in Spain (19), 36.5% in Belgium (48), and 36% in Korea (15). However, the rate was 60% in Portugal (5). In general, with the exception of Portugal, this lower rate of contamination may also be related to climate and temperature of food storage. All those countries with lower isolation rates are in Europe and northern Asia, which have temperate climates, whereas Ho Chi Minh City is tropical with a significantly higher average temperature, which may lead to replication of *Salmonella* spp. on carcasses and hence an increased likelihood of isolation from contaminated carcasses as well as a greater likelihood of exceeding the infection dose. Similarly, the reported rates of *Salmonella* contamination in pork and beef were also higher in developing countries compared to developed countries (3, 23, 24, 38, 43, 57). Different sampling procedures, sample types, and bacterial isolation and identification methods could affect the detected prevalences of *Salmonella* spp (8, 30, 39, 48). In addition, better equipment in slaughterhouses, advanced processing practices (including the use of dry chilling of carcasses), and more effective use of refrigeration in meat transport in developed countries could also help to reduce cross contamination of meats.

In general, our findings are similar to those of other studies in many countries showing that *Salmonella* isolates in retail meats was commonly resistant to TET, AMP, sulfonamides, and STR (12, 28, 42, 54, 55, 56). This reflects the use of these antibiotics in animal husbandry in many countries. In addition to these antibiotics, the results also showed that resistance of *Salmonella* isolates to NAL, the narrow-spectrum quinolone, was particularly high in Vietnam. Resistance to CIP and NOR was absent in all strains; however, resistance to ENR was observed. Fluoroquinolones are also a common choice of treatment for infections due to *Salmonella* spp. in humans (13, 26), and resistance to NAL may impair fluoroquinolone therapy (11, 44, 53). Therefore, the high level of NAL resistance in this study is of special concern as it may lead to the loss of therapeutic usefulness of fluoroquinolone. In addition, resistance to antibiotics such as AMP/AMX, CHL, and TET was often observed. These antimicrobial agents are still used widely in human therapy in Vietnam due to the low cost and ready availability (36). Therefore, resistance to these antibiotics in food-borne pathogens may create problems for disease treatment. The prudent use of antimicrobials in animal husbandry is therefore critical. Multiresistance occurred in potential human-pathogenic *Salmonella* serovars, including serovars Typhimurium, Albany, Anatum, Havana, and London. Multiresistant phenotypes were observed in most serogroup E isolates, of which the most frequent was serovar Anatum. Furthermore, one serovar Typhimurium pork isolate was resistant to eight antibiotics, and a serovar Albany isolate from chicken was resistant to seven antibiotics. These *Salmonella* serovars have also been isolated from clinical human isolates in Vietnam (52). There may be major health problems if these multiresistant isolates are transferred to humans.

The variant SGI1-F antibiotic resistance gene cluster was detected in *Salmonella* serovar Albany isolated from chicken meat, in which *aadA2* gene, which confers resistance to STR in serovar Typhimurium DT104, has been replaced by *dfrA1* (TMP resistance) and *orfC* (of unknown function). Gene exchange by homologous recombination could have occurred in this case to enable this change (31). This variant has previously been found in serovar Albany isolated from chicken in The Netherlands (51) and from fish meat in Thailand which was imported into France (20). In addition, this SGI1 variant has also been detected in other serovars, including Cerro and Dusseldorf, in infected patients recorded as having acquired the organism from Thailand and Malaysia, respectively (31). The identification of the SGI1 cluster in a variety of serovars, from different animal species and from humans in different countries, together with other characteristics of SGI1 such as the imperfect direct repeats flanking SGI1 in the *Salmonella* chromosome, suggests the SGI1-borne integrase, excisionase, and conjugative functions potentiate horizontal transfer (2, 7, 10, 22, 34). It is possible that this resistance cluster could be transferred to more acute human pathogens such as *Salmonella* serovar Typhi, further reducing the availability of effective antimicrobial therapy to control typhoid fever in Vietnam.

The results of this study have illustrated the extent of antibiotic resistance in *Salmonella* food-borne pathogens in raw food samples. This is the first report of an SGI1 variant from food samples in Vietnam. It is necessary to pay more attention to food hygiene practices to reduce or eliminate the risk from antibiotic-resistant and pathogenic bacteria originating from food. In addition, the use of antibiotics in animal feed needs to be regulated strongly to minimize the opportunity for organisms to develop resistance.

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