Detection of class 1 integron-associated gene cassettes of multi-drug resistant *Salmonella* strains isolated from food at conventional markets in Ho Chi Minh city

Truong Huynh Anh Vu[1*], Nguyen Hoang Khue Tu[3], Huynh Yen Ha[1], Chu Van Hai[2]

[1]Microbiology laboratory, Center of Analytical Services and Experimentation HCMC (CASE), Ho Chi Minh city, Vietnam,

[2]Department of Science and Technology HCMC, Ho Chi Minh city, Vietnam

[3]School of Biotechnology, Ho Chi Minh city International University, Vietnam National University Ho Chi Minh city, Vietnam

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Abstract

After *Salmonella* strains were isolated from food samples collected randomly at conventional markets in several districts in Ho Chi Minh city, we evaluated antibiotic resistance by Kirby-Bauer methods, and the serotype name was assigned according to ISO/TR 6579-3:2014 and class 1 integron was investigated by PCR technique. As a result, there were seven distinguished serovars, including *S.* Kentucky (8 strains); *S.* Infantis (4 strains); *S.* Agona and *S.* Potsdam (2 strains); *S.* Saintpaul, *S.* Braenderup, *S.* Indiana (01 strain); OMF:1,z_6:UT and 7:1,z_6:UT (01 strain). The rate of multidrug-resistance Salmonella serovars carrying class 1 integron was 100% (21/21). The gene cassette region of class 1 integron accounted for 85.71% (18/21). The presence of mobile genetic factors in *Salmonella* in the study suggests that the bacteria can transmit or receive antibiotic resistance genes from other bacterial species in the natural environment. In addition, the research results provide scientific evidence for management decisions and raise awareness of effective antibiotic use in Ho Chi Minh City, Vietnam.

Keywords: *Multidrug-resistance, antimicrobial resistance, Salmonella, integron, gene cassette.*

1. INTRODUCTION

Integron is a mobile genetic factor that plays an important role in the spread of antibiotic resistance genes in Gram-negative bacteria, especially they are common in bacteria of the Enterobacteriaceae family [1]. Integron has the ability to recognize and capture 1 or more gene cassette regions, usually containing genes encoding antibiotic

[*] *Corresponding author: Tel +84 909182442 Email: vutha@case.vn*

resistance [2]. Therefore, bacteria-carrying integrons often are multi-antibiotic resistant [3]. The general structure of the integrons consists of a conserved functional region 5'CS carrying components necessary for the system to function and a variable region containing many cassette genes encoding antibiotic resistance. The functional region of the integron includes three important sites: the site carrying the tyrosine recombinase enzyme (IntI) gene, which catalyzes the cleavage process and directs the binding of the integron of the cassette genes, and the specific binding site of the attI cassette gene and promoter site (Pc) [4]. Integrons themselves cannot move, but they are often associated with other movable genetic elements such as jumping genes or conjugate plasmids, whereby they can spread antibiotic resistance genes within and between species [5]. So far, at least 5 groups of mobile integrons have been identified, of which group 1 and group 2 integrons are commonly present in bacteria with multi-resistant phenotypes. They attract the attention of scientists because of their ability to spread resistance genes that can occur within the same species and between species [6]. This study was conducted to (i) identify serovars of *Salmonella* with multi-antibiotic resistance; (ii) study on the presence of group 1 integron along with the cassette gene regions of multi-antibiotic resistant serovars isolated from food at conventional markets in Ho Chi Minh City.

The research results will provide a scientific basis for future studies about the mechanism of multi-antibiotic resistance of *Salmonella* spp. at the molecular level isolated from food. In addition, the study supplements scientific evidence for state decisions on management and raising awareness of effective antibiotic use in Vietnam in general and in Ho Chi Minh City in particular.

2. MATERIALS AND METHODS

2.1. Objective

150 strains of *Salmonella* spp. isolated from food at traditional markets in Ho Chi Minh City are kept in Cryobank tubes at -70°C at the Center for Analysis and Experimental Services of Ho Chi Minh City.

2.2. Method

2.2.1. Evaluation of antibiotic resistance ability of Salmonella spp.

Take 3 - 5 pure colonies of each strain of *Salmonella* spp. on Nutrient Agar (Merck/ 1.05450) to perform antibiotic susceptibility assessment by Kirby-Bauer method on Muller Hinton Agar (Oxoid/CM0337). Based on the inhibitory zone diameter according to CLSI guidelines (2018) [7] interpret the results of antibiotic sensitivity (R/I/S) of Salmonella.

The antibiotics used in this study were selected according to Decision 2625/QD-BNN-TY [8]. Sterile antibiotic discs (Oxoid, UK) with a diameter of 6 mm were impregnated with antibiotic solutions with the following concentrations: ampicillin (AM, 10 µg/mL), amoxicillin/clavulanic acid (AMC, 30 µg/mL), ceftazidime (CAZ, 30 µg/mL), chloramphenicol (C, 30 µg/mL),

ciprofloxacin (CIP, 5 µg/mL), ofloxacin (OFX, 5 µg/mL), gentamicin (CN, 10 µg/ mL), streptomycin (STR, 10 µg/mL), nalidixic acid (NA, 30 µg/mL), tetracycline (TE, 30 µg/mL), and sulfamethoxazole/trimethoprim (SXT, 30 µg/mL).

2.2.2. Total DNA extraction method

Using a circular culture rod, take a colony ring on Nutrient Agar (Merck/1.05450) and place in Eppendorf containing 1 mL of sterile distilled water. DNA extraction was performed using the AccuRive Bacteria DNA Prep Kit (KT Biotech) (https://kt-biotech.com/san-pham/accurive-bacteria-dna-prep-kit).

2.2.3. Detection group 1 integron and cassette gene regions by PCR

For multi-antibiotic resistant Salmonella serovars isolated from food, the presence of integron groups [9] and the cassette gene region were determined based on the use of corresponding specific primer pairs by PCR [10]. The sequences of primer pairs and amplification product sizes are shown in Table 1.

m-PCR reaction components include 2 UI AmpliTaq Gold; 0.2 mM dNTP; 1.5 mM $MgCl_2$; buffer 1X; 0.5 µL of each primer (concentration of 0.625 µM); 5 µL of template DNA and 25 µL of deionized distilled water, amplification program on Mastercycler (Eppendorf) was as follows:

For the determination of integron group 1: 94°C/5 min (1 cycle); 94°C/60s; 60°C/30s and 72°C/60s (30 cycles); 72°C/05 min (1 cycle).

Positive control: *Salmonella enterica* subsp. *enterica* serovar Kentucky 1600.

For the detection of cassette genes: 94ºC/05 min (1 cycle); 94ºC/01 min, 58ºC/02 min, 72ºC/02 min (35 cycle); 72ºC/10 min (1 cycle).

PCR products were determined by electrophoresis-UV on 1% agarose agar at 100V for 30 min.

2.2.4. Electrophoresis method

PCR products were electrophoresed on a 1.5% agarose gel containing 1 µg/mL ethidium bromide in TBE. Ladder ladders were also electrophoresed simultaneously. Electrophoresis time was 35 - 40 min at 100 V and 100 mA. The gel was then photographed with UV light using an Ingenius gel camera.

***Table 1**. Primer sequence used for PCR reaction*

Target	***Primer***	***Sequence 5'-3'***	***Size (bp)***	***Ref***
Integron group 1	*Int*1 F	CAGTGGACATAAGCCTGTTC	164	[9]
	*Int*1 R	CCCGAGGCATAGACTGTA		
Cassette integron 1	*5'CS*	GGCATCCAAGCAGCAAG	Variable*	[10]
	3'CS	AAGCAGACTTGACCTGA		

3. RESULTS AND DISCUSSIONS

3.1 Antibiotic resistance phenotype of *Salmonella* strains

From the results of the antibiogram of 150 strains of Salmonella isolated from food, we have determined the multi-antibiotic resistance phenotype of 21 strains of *Salmonella* resistant to 07 antibiotics or more, this result was presented in Table 2. Thereby, it showed that *Salmonella* strains isolated from fish samples have more antibiotic-resistant phenotypes than other samples, specifically AMP, C, NA, GM, STR, TE, SXT (04 strains), AMP, C phenotypes. , NA, CIP, OFX, GM, STR, TE (02 strains) and AMP, C, NA, CIP, OFX, GM, STR, TE, SXT (03 strains), followed by *Salmonella* isolated from chicken samples have antibiotic phenotypes AMP, CAZ, C, NA, CIP, OFX, GM, STR, TE, SXT (02 strains); AMP, C, NA, GM, STR, TE, SXT (02 strains). Particularly, 03 strains with symbols SA07/20 1066, SA07/20 1067 derived from chicken, and SA11/19 3514 from fish have the same antibiotic resistance phenotype: AMP, CAZ, C, NA, CIP, OFX, GM, STR, TE, SXT.

Table 2. *Multi-antibiotic resistance phenotype of Salmonella spp.*

Source	***Strain code***	***Antibiotic resistance phenotype***	***Number of strain***
Pork	SA11/19 3497	AMC, AMP, C, NA, CIP, OFX, STR, TE	2/21
	SA11/19 4221	CAZ, C, NA, CIP, OFX, GM, STR, TE, SXT	
Beef	SA07/20 3335	AMP, C, NA, CIP, OFX, STR, TE, SXT	1/21
Chicken meat	SA11/19 3498	AMC, AMP, CAZ, C, STR, TE, SXT	5/21
	SA12/19 1584	AMP, C, NA, GM, STR, TE, SXT	
	SA05/20 1114	AMP, C, NA, GM, STR, TE, SXT	
	SA07/20 1066	AMP, CAZ, C, NA, CIP, OFX, GM, STR, TE, SXT	
	SA07/20 1067	AMP, CAZ, C, NA, CIP, OFX, GM, STR, TE, SXT	

Source	*Strain code*	*Antibiotic resistance phenotype*	*Number of strain*
Fish	SA11/19 3514	AMP, CAZ, C, NA, CIP, OFX, GM, STR, TE, SXT	13/21
	SA11/19 3515	AMP, C, NA, CIP, OFX, GM, STR, TE, SXT	
	SA11/19 4205	AMP, C, NA, CIP, OFX, GM, STR, TE, SXT	
	SA12/19 501	AMP, C, NA, CIP, OFX, GM, STR, TE	
	SA12/19 1600	AMP, C, NA, CIP, OFX, GM, STR, TE	
	SA01/20 66	AMP, C, NA, GM, STR, TE, SXT	
	SA02/20 1524	AMP, C, NA, GM, STR, TE, SXT	
	SA05/20 210	AMP, C, NA, GM, STR, TE, SXT	
	SA06/20 1808	AMP, C, NA, CIP, GM, STR, TE, SXT	
	SA06/20 1809	AMP, NA, CIP, GM, STR, TE, SXT	
	SA07/20 460	AMP, C, NA, CIP, GM, STR, TE	
	SA07/20 462	AMP, C, NA, CIP, OFX, GM, STR, TE, SXT	
	SA08/20 2058	AMP, C, NA, GM, STR, TE, SXT	

Note: AMC (Amoxicillin/ Clavunic acid), AM (Ampicillin), C (Chloramphenicol), NA (Nalidixic acid), CIP (Ciprofloxacin), OFX (Ofloxacin), GN (Gentamycin), STR (Streptomycin), TE (Tetracycline), SXT (Sulfamethoxazole/Trimethoprim)

3.2 Results of serovar determination of *Salmonella* spp. multi-antibiotic resistance

From the results of determining the multidrug resistance phenotype, serovar determination of 21 *Salmonella* strains was performed according to ISO/TR 6579-3:2014 using antisera O and H by agglutination reactions on glass slides and in vitro [11]. The results in Table 3 showed that 07 serovars have been identified, of which the most is Kentucky serovar (8 strains); Infantis (4 strains); Agona and Potsdam (2 strains); remaining each strain

for the serovar Saintpaul, Braenderup, Indiana. However, for two *Salmonella* strains with symbols SA07/20 460 and SA07/20 462 isolated from seafood samples, serovar could not be recognized (during the H antigen agglutination test, the process phase transition did not occur) so only the antisera formulas were determined as *OMF:1,z_6:UT* and *7:1,z_6:UT*.

Table 3. *Results of serovar determination of Salmonella spp. multi-antibiotic resistance*

Source	***Strain code***	***Antigen formula***			***Formula***	***Serovar***
		O antigen	***H antigen***			
			Phase 1	***Phase 2***		
Pork	SA11/19 3497	8	i	1,z_6	8:i:1,z6	*S.* Kentucky
	SA11/19 4221	4	z	1,7	4:1,7:z	*S.* Indiana
Beef	SA07/20 3335	7	r	1,5	7:1,5:r	*S.* Infantis
Chicken meat	SA11/19 3498	4	f,s,g		4:f,s,g	*S.* Agona
	SA12/19 1584	7	r	1,5	7:1,5:r	*S.* Infantis
	SA05/20 1114	7	l,v	en,z_{15}	7:L,v:en,z_{15}	*S.* Potsdam
	SA07/20 1066	8	i	1,z_6	8:i:1,z_6	*S.* Kentucky
	SA07/20 1067	8	i	1,z_6	8:i:1,z_6	*S.* Kentucky
	SA11/19 3514	8	i	1,z_6	8:i:1,z_6	*S.* Kentucky
	SA11/19 3515	4	eh	1,2	4:eh:1,2	*S.* Saintpaul
	SA11/19 4205	7	e,h	e,n,z_{15}	7:e,h:e,n,z_{15}	*S.* Braenderup
	SA12/19 501	8	i	1,z_6	8:i:1,z_6	*S.* Kentucky
	SA12/19 1600	8	i	1,z_6	8:i:1,z_6	*S.* Kentucky
	SA01/20 66	7	l,v	en,z_{15}	7:L,v:en,z_{15}	*S.* Potsdam
Fish	SA02/20 1524	8	i	1,z_6	8:i:1,z_6	*S.* Kentucky
	SA05/20 210	7	r	1,5	7:1,5:r	*S.* Infantis
	SA06/20 1808	4	f,s,g		4:f,s,g	*S.* Agona
	SA06/20 1809	7	r	1,5	7:1,5:r	*S.* Infantis
	SA07/20 460	OMF	1,z_6		OMF:1,z_6:UT[a]	-
	SA07/20 462	7	1,z_6		7:1,z_6:UT[a]	-
	SA08/20 2058	8	i	1,z_6	8:i:1,z_6	*S.* Kentucky

Note: [a]: No classification

The results also showed that 100% of serovar were resistant to STR and TE. The antibiotic with the least serovar resistance rate was AMC 9.52% (2/21), followed by CAZ 23.81% (5/21). This shows that AMC and CAZ antibiotics are still effective against isolated multi-resistant serovars. *S.* Indiana, *S.* Infantis, *S.* Saintpaul, *S.* Braenderup had AMC

sensitivity rate of 100.0%, *S.* Agona 50.0%, *S.* Kentucky 5.26%. Particularly, *S.* Kentucky has the symbol 07/20 1066; 07/20 1067 and 12/19 1600 had the highest number of resistant antibiotics (10 types).

Table 4. *Number of multi-antibiotic resistant serovars sorted by source*

Serovar	***Pork (n = 2)***		***Beef (n = 1)***		***Chicken meat (n = 5)***		***Fish (n = 13)***	
	Number	***Rate (%)***	***Number***	***Rate (%)***	***Number***	***Rate (%)***	***Number***	***Rate (%)***
S. Kentucky	01	50,0	-	-	02	40,0	05	38,46
S. Indiana	01	50,0	-	-	-	-	-	-
S. Infantis	-	-	01	100,0	01	20,0	02	15,38
S. Agona	-	-	-	-	01	20,0	01	7,69
S. Saintpaul	-	-	-	-	-	-	01	7,69
S. Braenderup	-	-	-	-	-	-	01	7,69
S. Potsdam	-	-	-	-	01	20,0	01	7,69
OMF:1,z$_6$:UT[a]	-	-	-	-	-	-	01	7,69
7:1,z$_6$:UT[a]	-	-	-	-	-	-	01	7,69
Tổng (n = 21)	2/21	9,52	1/21	4,76	5/21	23,81	13/21	61,90

In addition, the study results noted that *Salmonella* spp. from fish samples with the highest number of multi-antibiotic resistant serovars, 61.90% (13/21 strains), followed by chicken strains 23.81% (5/521 strains), pork 9.52% (2/21), finally beef 4.76% (1/21). *S.* Kentucky was the predominant serovar in all three sources (pork: 01; chicken: 02; fish: 05 strains), only *Salmonella* from beef did not detect Kentucky serovar (Table 4). The results partly reflect that the situation of multi-antibiotic-resistant *Salmonella* isolated from fish, chicken, and pork meat sold at traditional markets in Ho Chi Minh City is alarming and warning to consumers. On the other hand, the stronger participation of all levels of management is really urgent in the current period.

3.4. Detection results of integron and cassette gene regions

The results in Table 5 show that group 1 integrons was found in all multi-antibiotic resistant Salmonella serovars isolated from food groups. Group 1 integrons are commonly present in multidrug-resistant bacteria and are detected in many Gram-negative bacteria [1]. In addition, their presence can significantly contribute to the horizontal gene transfer of resistance genes between bacterial species from different sources or geographical regions.

The amplification results with primers 5'CS and 3'CS showed that the cassette gene region was detected in 18/21 Salmonella strains (85.71%) with 08 different sizes (> 1.0 kbp; 1.0). kbp; 0.9 kbp; 0.6 kbp; 0.5 kbp; 0.4 kbp; 0.25 kbp; 0.2 kbp). The most amplified region is 0.6 kbp (9/21, 42.86%), followed by 1.0 kbp (7/21, 33.33%), 0.25 kbp (6). /21, 28.57%), 0.9 kbp (5/21, 23.81%), > 1.0 kbp (4/21, 19.05%) 0.4 and 0.2 kbp (2/ 21, 9.53%) and the lowest is 0.5 kbp (1/21, 4.76%). The proportion of Salmonella serovars that did not carry the cassette gene region of integron 1 was 14.29% (3/21), carrying 4 regions at the same time was 4.76% (1/21), 3 and 2 regions are 23.81% (5/21), 1 region 33.33% (7/21). The cassette gene region > 1.0 kbp was mainly present in Kentucky serovars with a rate of 50% (4/8), these serovars all shared the same phenotype of resistance to β-lactam antibiotics and aminoglycosides.

All serovars containing the 0.9 and 1.0 kbp integron cassette gene regions of group 1 were resistant to quinolones, tetracyclines, and sulfonamides. From the analyzed data, we determined that there is a correlation between the integron containing the cassette gene regions and the resistance to AM, NA, CIP, SXT, STR, GM, and TE of the serovars isolated from food. This has also been explained by previous studies, most of the cassette gene regions bind to integron 1 of many bacterial species carrying genes for resistance to β-lactams, quinolones, aminoglycosides, and tetracyclines [6, 12]. However, the difference in the occurrence rate, as well as the combination and arrangement of genes in the gene cassette region in bacteria, has so far not been proven. Therefore, further studies need to be done to elucidate this issue.

Table 5. *Presence of cassette gene regions belonging to integron 1 of Salmonella*

Source	***Strain code***	***Serovar***	***Int 1***	***Cassette gene region size (kbp)***
Pork	SA11/19 3497	*S.* Kentucky	+	-
	SA11/19 4221	*S.* Indiana	+	0,25
Beef	SA07/20 3335	*S.* Infantis	+	1,0; 0,9
Chicken meat	SA11/19 3498	*S.* Agona	+	0,25
	SA12/19 1584	*S.* Infantis	+	1,0
	SA05/20 1114	*S.* Potsdam	+	1,0; 0,6; 0,25
	SA07/20 1066	*S.* Kentucky	+	> 1,0; 0,6
	SA07/20 1067	*S.* Kentucky	+	> 1,0; 0,6; 0,4
Fish	SA11/19 3514	*S.* Kentucky	+	-
	SA11/19 3515	*S.* Saintpaul	+	0,2
	SA11/19 4205	*S.* Braenderup	+	1,0; 0,2

Source	*Strain code*	*Serovar*	*Int 1*	*Cassette gene region size (kbp)*
	SA12/19 501	*S.* Kentucky	+	> 1,0
	SA12/19 1600	*S.* Kentucky	+	-
	SA01/20 66	*S.* Potsdam	+	2,5
	SA02/20 1524	*S.* Kentucky	+	0,6; 0,4
	SA05/20 210	*S.* Infantis	+	1,0; 0,9; 0,6
	SA06/20 1808	*S.* Agona	+	0,6; 0,25
	SA06/20 1809	*S.* Infantis	+	1,0; 0,9; 0,5
	SA07/20 460	OMF:1,z_6:UT	+	0,6
	SA07/20 462	7:1,z_6:UT	+	1,0; 0,9; 0,6; 0,25
	SA08/20 2058	*S.* Kentucky	+	> 1,0; 0,9; 0,6

4. CONCLUSION

Research has shown the multi-antibiotic resistance of Salmonella serovars and an increasing trend with multiple antibiotics. In addition, we detected the presence of group 1 integron in 100% of multi-antibiotic-resistant *Salmonella* along with 08 different cassette gene regions in 85.71% of the total strains. The research results are intended to provide data for scientists to conduct further studies on the mechanism of multi-antibiotic resistance at the molecular level. At the same time, investigating the presence of other groups of integrons, and studying the characteristics of the gene cassettes of Salmonella isolated from food is also necessary and worthy of attention.

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Sự hiện diện integron nhóm 1 và vùng gen cassette ở các chủng *Salmonella* đa kháng kháng sinh phân lập từ thực phẩm tại các chợ truyền thống trên địa bàn thành phố Hồ Chí Minh

Trương Huỳnh Anh Vũ[1], Nguyễn Hoàng Khuê Tú[3], Huỳnh Yên Hà[1], Chu Vân Hải[2]

[1]Phòng Vi sinh, Trung tâm Dịch vụ Phân tích Thí nghiệm thành phố Hồ Chí Minh, Việt Nam

[2]Sở Khoa học và Công nghệ thành phố Hồ Chí Minh, Việt Nam

[3]Bộ môn Công nghệ Sinh học, Trường Đại học Quốc tế, Đại học Quốc gia thành phố Hồ Chí Minh, Việt Nam

Tóm tắt

Chúng tôi tiến hành đánh giá đặc điểm nhạy cảm kháng sinh bằng phương pháp khuếch tán trong thạch (Kirby-Bauer) đối với 150 chủng *Salmonella* phân lập từ các mẫu thực phẩm thu thập tại các chợ truyền thống trên địa bàn thành phố Hồ Chí Minh. Sau đó 21 chủng *Salmonella* có khả năng kháng từ 07 loại kháng sinh trở lên được lựa chọn để xác định kiểu huyết thanh (serovar) theo phương pháp ISO/TR 6579-3:2014, sự hiện diện integron nhóm 1 và các vùng gen cassette được khảo sát bằng kỹ thuật PCR. Kết quả nghiên cứu đã định danh được 07 nhóm serovar khác nhau, *S.* Kentucky (8 chủng); *S.* Infantis (4 chủng); *S.* Agona *và S.* Potsdam (2 chủng); *S.* Saintpaul, *S.* Braenderup, *S.* Indiana (1 chủng); 2 chủng không xác định được serovar với công thức kháng huyết thanh là *OMF:1,z_6:UT* và *7:1,z_6:UT*. Tỷ lệ các serovar *Salmonella* đa kháng kháng sinh mang integron nhóm 1 là 100%, trong đó, các vùng gen cassette được phát hiện ở 85,71% tổng số chủng. Sự hiện diện của các yếu tố di truyền vận động ở *Salmonella* trong nghiên cứu cho thấy khả năng vi khuẩn này có thể truyền hoặc nhận gen kháng kháng sinh từ các loài vi khuẩn khác trong môi trường tự nhiên là rất cao. Bên cạnh đó, kết quả nghiên cứu góp phần cung cấp bằng chứng khoa học cho các quyết định cấp nhà nước về quản lý và nâng cao ý thức sử dụng kháng sinh có hiệu quả tại Việt Nam nói chung và thành phố Hồ Chí Minh nói riêng.

Từ khóa: *đa kháng kháng sinh, Salmonella, integron, vùng gen cassette.*