

THE RATE OF *SALMONELLA SPP.* FROM HEALTHY PEOPLE IN HO CHI MINH CITY IN 2018

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Abstract

Survey results of 226 stool samples from healthy people collected from four districts in Ho Chi Minh city in 2018 (districts no. 1, 5, 8 and 9) showed that the number of people carrying *Salmonella spp.* was 12/226 (5.3%). Twelve fecal samples were positive for *Salmonella spp.* with 15 *Salmonella spp.* strains isolated. Three of them are *S. Indiana* (20.0%); *S. Typhimurium*, *S. Rissen* and *S. Give*. Each has two strains (13.3%): 1 strain has serotypes O: 1 and one strains has 9: 1.5 (6.7%). These *Salmonella* strains belong to the non-typhoid group (the group that causes diarrhea).

The *Salmonella spp.* strains isolated have highest antibiotic resistance to ampicillin, tetracycline, chloramphenicol (80.0%); followed by sulfamethoxazole/trimethoprim (66.7%); nalidixic acid (40.0%); gentamicin, ciprofloxacin, kanamycin (26.7%). Strains resistant to the third generation cephalosporins were found (cefotaxime and ceftazidime). Three of these are resistant to both types of antibiotics (20.0%). No strains resistance to cefoxitin and cosfomycin were detected.

Keywords: *Salmonella spp.*, Ho Chi Minh City.

1. INTRODUCTION

Salmonella spp. is normally presented in livestock and poultry products such as eggs and milk. People eating these products may be at risk of being infected with *Salmonella spp.* strain as well as carrying the pathogen, thus spreading it out into the land, water source and food stuff. So if rules of food safety are not strictly respected, healthy people carrying *Salmonella* could be a hazard to the social community. With 18.5% as the rate of infection (from the national objectives program about food safety in the period from 2011 to 2015 (March/2017-Hanoi)) of *Salmonella spp.* in numerous types of food, this is one of four important reasons for diarrhea from micro-organisms. According to Vietnam's Ministry of Health, from January to November 2018, there were 547 diarrhea cases diagnosed as Salmonellosis.

In order to investigate the rate of healthy people containing *Salmonella spp.* as well as assess the antibiotic resistance in Ho Chi Minh City, the survey was polled in four representative districts including districts no. 1, 5, 8 and 9 which differed from economic status, location and living condition. This study was taken with two main targets:

Find out the rate of healthy people carrying *Salmonella spp.* and the serotypes of *Salmonella spp.* in districts no. 1, 5, 8 and 9 of Ho Chi Minh city.

Find out the rate of antibiotic resistance of the strains isolated.

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2. MATERIALS AND METHOD

+ **Method:** cross-sectional study.

+ **Time and location**

Time: from 1/2018 to 6/2018.

Location: districts no. 1, 5, 8 and 9 of Ho Chi Minh City.

Sampling location: ward medical centers.

Testing centers: biology testing department, food safety quality control center in southern area, Institute of Public Health in Ho Chi Minh City

+ **Research objects:** Stool sample: randomly taken from healthy people living in sampling location at the age of 15 or above.

- **Sample size**

Sample size formula was employed to estimate the number of feces of healthy people.

$$n = \frac{(Z_{1-\frac{\alpha}{2}})^2 \times p(1-p)}{d^2}$$

Where: n: sample size, p: the expected rate, d: desired width of confidence interval, α : type I error, $Z_{1-\alpha/2}$: coefficient respective to α . According to Anders, Kl et al (2015), p was set at 0.18, thus n was 226.

+ **Cultures**

Selenite, XLD, TSI, LIM, TSB, SIM Media.

O and H antiserum (Denka Seiken Ltd., Tokyo, Japan; SSI Diagnostica, Hillerød, Denmark).

Antibiotic disk: antibiotics were used in treating diarrhea in healthy people infected with gram (-) of bacteria, also used in aquaculture and husbandry including ampicillin 10 μ g (AMP), tetracycline 30 μ g (TET), kanamycin 30 μ g (KAN), chloramphenicol 30 μ g (CHL), centamicin 10 μ g (GEN), trimethoprim 23.75 μ g and sulfamethoxazole 1.25 μ g (SXT), Ciprofloxacin 5 μ g (CIP), nalidixic acid 30 μ g (NAL), fosfomycin (FOF) 20 μ g, cefoxitin 30 μ g (FOX), cefotaxime 30 μ g (CTX) and ceftazidime 30 μ g (CAZ). Combination of antibiotic disc CTX 30 μ g + clavulanic acid 10 μ g and CAZ 30 μ g, and CAZ 30 μ g + clavulanic acid 10 μ g (BD, Sparks, MD).

+ **Methods**

+ Employ survey questionnaire to determine background information of the target;

+ Stool sample: collected from healthy people by using a cotton swab, anal swab and stored in Cary-Blair environment, stored cool and transported to the laboratory for testing *Salmonella* spp. according to the standard procedure for culturing and isolating *Salmonella* spp [12];

+ Antiserum of *Salmonella* spp was determined according to WHO Collaborating Center for Reference and Research on *Salmonella*, 2007 [2];

+ Antibiotic resistance of *Salmonella* spp was determined by Kirby-Bauer technique and cross-resistance was assessed according to the Clinical and Laboratory Standards Institute (CLSI), 2017 [3].

3. RESULTS

3.1. The rate of *Salmonella* spp contamination

Table 1. The rate of *Salmonella* spp isolated from stool samples of healthy people

<i>Stool containing Salmonella spp.</i>	<i>Frequency</i>	<i>Percentage (%)</i>
Positive	12	5.3
Negative	214	94.7

The result of *Salmonella* isolation from 226 stool samples showed that 5.3% of sample contributed to positive results.

Table 2. The percentages of *Salmonella spp* contaminated

<i>District</i>	<i>The number of sample</i>	<i>Frequency</i>	<i>Percentage (%)</i>
No.1	50	2	4
No.5	60	2	3.3
No.8	60	2	3.3
No.9	56	6	10.7

The distribution of *Salmonella spp* isolated differs from district to district, the highest proportion of *Salmonella spp* contamination was found in district no. 9 (10.7%), the second highest was district no.1 (4%) and those of the other districts were at 3.3% each.

Table 3. The percentage of serum type of *Salmonella spp* strains isolation ($n = 15$)

<i>Type of serum</i>	<i>KN Formular</i>	<i>Frequency</i>	<i>Percentage (%)</i>
<i>S. Indiana</i>	9	3	20.0
<i>S. Rissen</i>	1.8	2	13.3
<i>S. Give</i>	9	2	13.3
<i>S. Typhimurium</i>	9	2	13.3
<i>S. Kentucky</i>	5	1	6.7
<i>S. London</i>	5	1	6.7
<i>S. Meleagridis</i>	9	1	6.7
<i>S. Weltevreden</i>	9	1	6.7
O: 1	8	1	6.7
9: 1, 5	1	1	6.7

In order to assess the origin of the isolated *Salmonella spp* strains, their types of serum were determined. Among 12 samples that were positive to *Salmonella spp*, there were 15 strains of *Salmonella spp* and 10 types of serum. In this study, there was one sample containing three strains of *Salmonella spp*, one sample containing two strains and the other 10 samples containing one strain.

3.2. Result of antibiotic resistance of *Salmonella spp* isolated

Table 4. The rate of antibiotic resistance of *Salmonella spp* isolated ($n= 15$)

<i>Antibiotic</i>	<i>Resistance</i>	
	<i>Frequency (n)</i>	<i>Percentage (%)</i>
Ampicillin (AMP)	12	80.0
Tetracycline (TET)	12	80.0
Chloramphenicol (CHL)	12	80.0
Sulfamethoxazole/rimethoprim (SXT)	10	66.7
Nalidixic acid (NAL)	6	40.0
Gentamicin (GEN)	4	26.7
Ciprofloxacin (CIP)	4	26.7



Kanamycin (KAN)	4	26.7
Cefotaxime (CTX)	3	20.0
Ceftazidime (CAZ)	3	20.0
Cefoxitin (FOX)	0	0
Fosfomycin (FOM)	0	0

In this study, 15 *Salmonella* strains had the highest antibiotic resistance to ampicillin, tetracycline, chloramphenicol (80%), followed by sulfamethoxazole/rimethoprim at 66.7%. This data for nalidixic acid was 40% and for gentamycin, ciprofloxacin and kanamycin were 26.7%. Lowest resistance was found against cefotaxime and ceftazidime at 20.0% and there was no strain resistant to cefoxitin and fosfomycin.

Table 5. The distribution of serum type from its antibiotic resistance phenotype of *Salmonella* spp isolated (n = 15)

Type of serum	Resistant phenotype	Frequency (Percentage %)
<i>S. Indiana</i>	AMP, TET, KAN, CHL, GEN, CIP, NAL, CTX, CAZ, SXT	3 (20.0)
<i>S. Kentucky</i>	AMP, TET, KAN, CHL, GEN, CIP, NAL, SXT	1 (6.7)
<i>S. London</i>	AMP, TET, CHL, SXT	1 (6.7)
<i>S. Rissen</i>	AMP, TET, CHL, SXT	1 (6.7)
<i>S. Typhimurium</i>	AMP, TET, CHL, SXT	2 (13.3)
<i>S. Meleagridis</i>	AMP, TET, CHL, SXT	1 (6.7)
<i>S. Weltevreden</i>	AMP, CHL, SXT	1 (6.7)
<i>S. Give</i>	TET, CHL, NAL	2 (13.3)
<i>S. Rissen</i>	AMP, TET	1 (6.7)
O: 1	AMP	1 (6.7)
9: 1.5		1 (6.7)
Summary		15

The result showed that there was one of the phenotypes of antibiotic resistance from *Salmonella* spp isolated from healthy human feces in four districts of Ho Chi Minh City (9:1.5) which was sensitive to all antibiotics in these experiments, while the multi-resistance strains (at least 3 antibiotic resistants) were at 80%. This study also proved that three strains of *S. Indiana* resisted to 10 kinds of antibacterials.

The phenotype ESBL of the *Salmonella* spp resistant to cefotaxime and ceftazidime was then determined by combined dish method. The results indicated that all of them were positive to ESBL, which can produce large spectrum β -lactamase and resisted to the third generation of cephalosporin.

4. DISCUSSION

Salmonella spp is one of the agents causing diarrhea in human. In recent years, there has been an increasing number of antibiotic-resistant strains of *Salmonella* spp, which is an important health issue in the world.

The percentage and types of serum of *Salmonella spp* isolated from stool samples of healthy people living in districts no. 1, 5, 8 and 9 in Ho Chi Minh City

Among 226 stool samples in four districts in Ho Chi Minh City, the percentage of *Salmonella spp* positive samples was 12/226 (5.3%), which was higher than those from other study conducted in Mekong Delta (3.3%) according to Trung NV et al [4]. This can be explained by the differences between economic conditions and living status of local residents.

In this study, the rate of *Salmonella spp* contaminations in four districts in Ho Chi Minh city was described. District no. 9 had the highest proportion of *Salmonella spp* contamination while the second highest data was found in district no.1. The reason may be derived from differences among living conditions, economic status of the and hygiene quality.

This is the first study about serotypes of *Salmonella spp* on healthy people carrying strains of *Salmonella spp* in Ho Chi Minh city. There were 15 strains of *Salmonella spp* isolated from 12 samples. There was only one *Salmonella spp* strain isolated from one sample in most of the cases. There were 10 people carrying one strain, one person carrying two strains and one person carrying three strains. Among 15 strains isolated, there were 10 different types of serum. *S. Indiana* accounting for 20%, then *S. Typhimurium* *S. Rissen* and *S. Give* (13.3%).

The isolated serotypes in this study were similar to serotypes isolated from fresh sustenance in Ho Chi Minh City in 2015 [5]. This result showed that the origin of food was responsible for healthy people carrying the strain of *Salmonella spp*. These serotypes belonged to non-typhoid group which is responsible for diarrhea.

The ratio of *Salmonella spp* resistance to antibiotics

The sensitivity of *Salmonella spp* was evaluated via isolation, multi-resistant phenotype (more than three antibiotics), the result was at 80% (12/15). Ampicillin, Tetracycline, Chloramphenicol accounting for 80%, Sulfamethoxazole/Trimethoprim was at 66.7%. These antibiotics is normally used in treating diarrhea [6], human diseases and livestock industry related to *Salmonella spp*. The sensitivity of Nalidixic acid accounted for 40%, that data for Ciprofloxacin was 26.7%. These results were higher than results from Trung et al (2017) (< 2%) [4]. All strains of *S. Indiana* which was resistant to 10 types of antibiotics including Ciprofloxacin. These results were similar to *Salmonella spp* isolated from pork, beef, poultry, fish and shrimp in Ho Chi Minh City [5]. The spread of multi-resistant antibiotics of *Salmonella spp* including Ciprofloxacin was a health issue for public in Ho Chi Minh city as well as Vietnam because these antibiotics were commonly used in disease related to *Salmonella spp* in humans.

In this study, there were three strains *S. Indiana* resistant to Cefotaxime and Ceftazidime, all of them were positively diagnosed with ESBL phenotype. The strains producing ESBL were detected around the world from food in Vietnam, and human in England [10]. Furthermore, the *Salmonella spp* strain producing ESBL normally co-resisted to Ciprofloxacin, which was a drawback in treatment [11]. This was a risk of spreading *Salmonella spp* from sustenances into healthy humans.

5. CONCLUSION

The rate of infection with *Samonella spp.* in healthy people in four districts in Ho Chi Minh City was at 5.3%, of which district no. 9 had the highest prevalence (10.7%) and the lowest was found in two districts no. 5 and no. 8 with the same infection rate at 3.3%. Serotypes of *Salmonella spp.*: *S. Indiana* consists of three strains (20.0%), followed by *S. Typhimurium*, *S. Rissen* and *S. Give* including two strains (13.3%), while other serotypes have one strain accounting for 6.7%.

The rate of antibiotic resistance of *Salmonella spp* strains isolated to antibiotics was as follows: Ampicillin, Tetracycline, and Chloramphenicol: 80.0%; Sulfamethoxazole/Trimethoprim: 66.7%; Nalidixic acid: 40.0%; Gentamicin, Ciprofloxacin, Kanamycin: 26.7%; There have been strains resistant to the third generation Cephalosporin: Cefotaxime and Ceftazidime, accounting for 20.0%.



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Tóm tắt

TỶ LỆ NGƯỜI KHỎE MẠNH MANG VI KHUẨN *SALMONELLA SPP.* TẠI THÀNH PHỐ HỒ CHÍ MINH NĂM 2018

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Viện Y tế Công cộng thành phố Hồ Chí Minh

Kết quả khảo sát 226 mẫu phân người khỏe mạnh thu thập từ 4 quận (quận 1, 5, 8 và 9) của thành phố Hồ Chí Minh cho thấy số người mang vi khuẩn *Salmonella spp.* là 12/226 (5,3%). Có 12 mẫu phân dương tính với *Salmonella spp.*, đã phân lập được 15 chủng *Salmonella spp.* Ba chủng là *S. Indiana* (20,0%); *S. Typhimurium*, *S. Rissen* và *S. Give*, mỗi loài có 2 chủng (mỗi loại 13,3%), 1 chủng có kiểu typ huyết thanh O 1 và 1 chủng 9: 1, 5 (mỗi loại 6,7%). Các chủng *Salmonella spp.* này thuộc nhóm nontyphi (nhóm gây tiêu chảy).

Tỷ lệ vi khuẩn *Salmonella spp.* đề kháng kháng sinh cao nhất là Ampicillin, Tetracycline, Chloramphenicol (80,0%); kế đến là Sulfamethoxazole/trimethoprim (66,7%); Nalidixic acid (40,0%); Gentamicin, Ciprofloxacin, Kanamycin (26,7%); đã xuất hiện chủng đề kháng với nhóm Cephalosporin thế hệ 3: Cefotaxime và Ceftazidime, có 3 chủng đề kháng 2 loại kháng sinh này chiếm tỷ lệ (20,0%); chưa có chủng đề kháng Cefoxitin và Fosfomycin.

Từ khóa: Người khỏe mạnh mang *Salmonella spp.* tại TP. Hồ Chí Minh