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Original Article

Molecular epidemiology and antimicrobial resistance of Salmonella species from clinical specimens and food Items in Lebanon

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Abstract

Introduction: Foodborne illnesses can be due to a wide range of bacteria, one of the most common being *Salmonella*. In this study, PulseNet International was implemented in Lebanon to identify circulating pathogens at the species and strain levels, determine antimicrobial resistance, and link food sources and clinical cases during outbreaks.

Methodology: Clinical and food *Salmonella* isolates received from the Epidemiological Surveillance Unit, Ministry of Public Health (ESUMOH) and the Lebanese Agriculture Research Institute (LARI) between 2011 and 2014 were identified to the species level using API 20E. Serotyping was carried out using the Kauffman and White scheme. Antimicrobial susceptibility to a panel of antimicrobials was tested by the disc diffusion method. The DNA fingerprinting patterns were determined using Pulsed-Field Gel Electrophoresis (PFGE) followed by BIONUMERICS analysis.

Results: 290 clinical and 49 food isolates were identified to be *Salmonella*. The serotyping of the isolates revealed the prevalence of ten serotypes in the clinical isolates and seven serotypes within the food isolates; *S.* Enteritidis and *S. Typhimurium* being the two most common. Antimicrobial susceptibility test showed resistance to tested antimicrobials among both clinical and food isolates. PFGE results showed a wide range of pulsotypes by the different serovars. These pulsotypes were then used to confirm the linkage of two outbreaks to their food sources.

Conclusion: This study calls out to set and implement food safety regulations and emphasizes the importance of surveillance through a "farm-to-fork" approach in identifying widely circulating food borne pathogens.

Key words: *Salmonella;* antimicrobial resistance; serotype; Pulsed-Field Gel Electrophoresis (PFGE); Lebanon; molecular epidemiology.

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Introduction

In spite of the advances in food safety surveillance and regulations, foodborne pathogens still cause 48 million cases of infections, 128,000 hospitalizations, and 3,000 deaths each year in the United States alone according to the Center of Disease Control and Prevention (CDC) [1]. Foodborne diseases arise due to the consumption of food products and beverages contaminated with bacteria, parasites, viruses, toxins, or chemicals [2]. These pathogens elicit symptoms pertaining mostly to the gastrointestinal tract such as diarrhea, nausea and vomiting. Other symptoms may include fever, malaise, headache and dizziness [3].

According to CDC, *Salmonella* resulted in 23% of outbreaks, 31% of diseases, and 62% hospitalization making it the second most common food borne pathogen [4]. S*almonella* spp. can be isolated from a wide range of food products such as fresh produce, ground beef, water, and ready-to-eat foods, even though it is mainly found in poultry and eggs [5]. There are more than 2500 known serotypes of *Salmonella*, which can be divided into two main groups: typhoidal serotypes which include *Salmonella Typhi* and *Salmonella Paratyphi* and nontyphoidal *Salmonella* which comprises a wide range of serotypes [6,7]. Typhoid and severe cases of gastroenteritis caused by *Salmonella* spp. have been treated with a wide range of antimicrobial agents including: broad spectrum antibiotics such as ampicillin, amoxicillin, tetracycline, and cotrimoxazole and fluoroquinolone such as ciprofloxacin [8]. Recently, *Salmonella* strains are becoming increasingly resistant to one or more antibiotics posing a public health concern [9].

In Lebanon, reporting foodborne illnesses by physicians is mandatory by the law on infectious diseases, issued on 31st January, 1957 [10]. A total of 1,747 cases including four deaths were reported to the Ministry of Public health (MoPH) between 2009 and 2013. Most reported suspected food products were ground raw meat (25%) and cooked chicken (11%). This was not unusual, since culturally the Lebanese food includes raw meat frequently consumed by people. During this period, *Salmonella* was the mostly isolated (32%) infectious agent (unpublished data from MoPH). Since reporting of foodborne pathogens from various laboratories in different areas in Lebanon can be incomplete and delayed, a collaboration between the MoPH and the American university of Beirut (AUB) began in the year 2009 establishing the disease tracking network, PulseNet International, in Lebanon. This collaboration includes public and private sectors working closely together to strengthen the surveillance of foodborne diseases by identifying the species and strains of the pathogens circulating nationwide, determining antimicrobial resistance patterns, and relating clinical cases to their food sources during outbreaks.

Methodology

Between 2011 and the beginning of 2014, *Salmonella* positive clinical isolates were identified and isolated from stool specimens of patients from different satellite clinical laboratory networks and hospitals nationwide. Additionally, food samples including a wide range of items were tested for bacterial agents and *Salmonella* positive isolates were identified at the Lebanese Agriculture Research Institute (LARI). These food and clinical isolates were collected by the Ministry of Public Health Epidemiological Surveillance Unit (ESUMOH) on a routine basis. Moreover, during outbreak investigations (as confirmed by the ESUMOH unit, MoPH), stool specimens obtained from patients that acquired the foodborne illness, were sent to the closest clinical laboratory and the corresponding suspected food samples were sent to LARI for testing and identification. Consequently, all isolates (sporadic and outbreak) were sent to the PulseNet laboratory, AUB, after which they were cultured on MacConkey (Scharlau Chemie, Sentmenat, Spain) agar and kept in Brucella broth (Becton, Dickinson & Co., Sparks Glencoe, USA) with 10% glycerol at -20ºC for long term storage. The isolates were identified using the API 20E kit (Biomérieux, Marcy L'etoile, France) and the results were analyzed later by the APIweb™ software. (Biomérieux, Marcy L'etoile, France).

Serotyping

Serotyping of the isolates was carried out according to the Kauffman and White scheme [11]. Initially the O antigen, the outermost portion of lipopolysaccharide, was determined followed by the H antigen, the flagellin protein [12], by the latex agglutination test using mono- and poly-valent anti-sera (Biorad, Hercules, USA).

Pulsed-Field Gel Electrophoresis (PFGE)

PFGE was performed using the Bio-Rad CHEF MAPPER (Biorad, USA) applying the standard operating procedure for PulseNet PFGE of *E. coli* O157:H7, *E. coli* non- O157 (STEC), *Salmonella* serotypes, *Shigella sonnei*, and *S. flexneri* [13] with the *XbaI* (Fermentas, Waltham, USA) as restriction endonuclease to determine their genomic relatedness. Gel Doc XR+ system Machine and "Quality one" software were employed to visualize the bands and capture a picture of the gel. The dendrograms were generated using the UPGMA method (unweighted pair group method using arithmetic averages) with the BIONUMERICS software (Applied Maths BVBA, Keirstraat, Belgium). The profiles were assigned codes made up of ten letter-digits in which the first three characters in the code symbolize the bacterial pathogen, the next three characters represent the enzyme used for DNA restriction, and the last four characters represent the pulsotype label.

Antimicrobial susceptibility testing- The disk diffusion method

Antimicrobial susceptibility testing (AST) using the Kirby-Bauer method was performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines [14]. Standard discs (Oxoid, Basingstoke, England) of antibiotics commonly used in the treatment of *Salmonella* infections were selected. These included ampicillin, trimethoprim-sulfamethoxazole, ciprofloxacin, and ceftazidime [15].

Results

Two hundread and ninety isolates of the clinical samples obtained from the ESUMOH and 49 isolates of the food samples obtained from LARI between 2011 and beginning of 2014 were re-identified as *Salmonella* spp. Serotyping revealed the prevalence of ten serotypes among the clinical samples and seven serotypes among the food *Salmonella* strains (Table 1). The common serotypes isolated in both clinical and food samples were: *Salmonella* Typhimurium, *Salmonella* Enteritidis, *Salmonella* Braenderup, *Salmonella* Typhi, *Salmonella* Paratyphi A, *Salmonella*

Table 1. Distribution of *Salmonella* serotypes isolated from clinical and food samples

API profile	Serotype	Total	
		Clinical	Food
Salmonella spp.	*Salmonella* Typhimurium	84(29.0)	14(28.5)
	Salmonella Enteritidis	126(43.4)	10(20.4)
	Salmonella Braenderup	21(7.2)	4(8.2)
	Salmonella Typhi	19(6.6)	3(6.1)
	Salmonella London	10(3.4)	-
	Salmonella Paratyphi A	8(2.8)	8(16.3)
	Salmonella Blockley	3(1.0)	2(4.1)
	Salmonella Paratyphi B	2(0.7)	-
	Salmonella Newport	2(0.7)	4(8.2)
	Salmonella Paratyhi C	2(0.7)	-
	Other	13(4.5)	4(8.2)
	Total	**290(100)**	**49(100)**

Blockley, and *Salmonella* Newport. Few of the samples did not show agglutination with the O and H anti-sera available in the lab and were termed "Others". The two most common serotypes isolated between 2011 and 2014 from clinical and food samples were *S. Enteritidis* (clinical = 43.4% and food = 20.4%) and *S. Typhimurium* (clinical, n = 29% and food, n = 28.5%) (Table 1).

Pulsed-Field Gel Electrophoresis (PFGE)

PFGE results showed a wide range of pulsotypes indicated by different DNA patterns present among the *Salmonella* serotypes. The analysis revealed the presence of 13 and seven pulsotypes among *S.* Typhimurium isolates recovered from clinical and food samples, respectively. Additionally, only two pulsotypes of *S.* Enteritidis were present in both food and clinical samples. The serotype *S.* Braenderup exhibited five PFGE profiles in the clinical isolates, and only one in the food isolates. On the other hand, *S.* Typhi showed the presence of four pulsotypes in the clinical samples and three pulsotypes in the food samples.

There were eight pulsotypes of *S.* London identified in the clinical samples. Moreover, *S.* Paratyphi A had a diverse distribution of pulsotypes within the clinical (six pulsotypes) and food isolates (eight pulsotypes). Two pulsotypes of *S.* Blockley were isolated from the clinical and food samples. Furthermore, there were four and one pulsotypes of *S.* Newport isolated from food and clinical samples, respectively. Both *S.* Paratyphi B and C had only one pulsotype in the clinical samples. Table 2 shows the total number and percentages of the different pulsotypes of the various serotypes in food and clinical samples, the most common pulsotype in each category, and the shared pulsotypes between the food and clinical samples.

Apart from identifying the pulsotypes circulating among the various serotypes, PFGE was able to link some outbreaks to their suspected food isolates. For example, during 2011, clinical and suspected food samples from two outbreaks of Salmonella in different areas of Lebanon were sent to the AUB lab. Serotyping and PFGE of these samples showed that the causative agent was: 1) Typhimurium with the pulsotype JPXX01.0002 in Nabatieh during February (clinical samples were isolated on 22 February 2011); raw meat being the food agent that caused the outbreak (isolated on 4 March, 2011). 2) *S.* Enteritidis pulsotype, JEGX01.0001, in Mount Lebanon in September (clinical samples were isolated on 22 September 2011); Arabic sweets were identified to be the source (isolated

Figure 1. The serotypes and pulsotypes of *Salmonella* spp. From food samples and clinical specimens isolated during outbreaks identified by Ministry of Public Health. AUB: American University of Beirut, PFGE: Pulsed-Field gel Electrophoresis.

Outbreak in Nabatieh, February 2011	
Patient stool culture	5 *Salmonella* spp.
Food samples	1 suspected samples of raw meat with *Salmonella* spp.
Serotyping in PulseNet Lab-AUB	*Salmonella* Typhimurium in 5 patient samples and 1 raw meat sample
PFGE type in PulseNet Lab-AUB	JPXX01.0002 for *Salmonella* Typhimurium patient and one raw meat sample
Outbreak in Mount Lebanon, September 2011	
Patient stool culture	*Salmonella* spp. in 8 patient samples
Food samples	*Salmonella* spp. in 2 samples of Arabic sweets
Serotyping in PulseNet Lab-AUB	*Salmonella* Enteritidis in 8 patient samples and 2 Arabic sweet samples
PFGE type in PulseNet Lab-AUB	JEGX01.0001 for *Salmonella* Enteritidis in patient samples and Arabic sweet samples

on 4 October 2011). The serotypes and pulsotypes of the food and clinical samples isolated during the outbreaks are shown in Figure 1. A representative of several dendrograms of *Salmonella* spp. from clinical samples matching those from food isolates is shown in Figure 2.

Antimicrobial susceptibility testing

Among the samples received, 73.8% of the clinical samples and 75.5% of the food samples were susceptible to the four antibiotics tested. All of the Salmonella isolates were resistant to one or more of the tested antimicrobial agents. Among the clinical samples, 23.4% (n = 68) exhibited resistance to ampicillin, 7.9% (n = 23) to trimethoprim-sulfamethoxazole, 3.8% (n = 11) to ciprofloxacin and 2.4% (n = 7) to ceftazidime. Among the food strains, 20.4% (n = 10) showed resistance to ampicillin, 10.2% (n = 5) to ciprofloxacin, and 2% (n = 1) to ceftazidime. However, they were all susceptible to trimethoprim-sulfamethoxazole.

Salmonella Typhi had the highest and next to the highest percentage of isolates in the clinical samples resistant to 1) ampicillin (57.9%) and ceftazidime (5.3%) and 2) trimethoprim-sulfamethoxazole (42.1%), respectively. Of the resistant *S.* Typhi pulsotypes, JPPX01.0001 had the highest reistance. *S.* Paratyphi B and *S.* Newport possessed the highest percentage (50%) of isolates resistant to ciprofloxacin while *S.* Paratyphi B had the highest percentage (50%) of isolates resistant to trimethoprim-sulfamethoxazole. The resistant pulsotype of *S.* Newport was JJPX01.0001 while that of *S.* Paratyphi B was unspecified.

Concerning the food samples *S.* Enteritidis and *S.* Newport had the highest percentage (50%) of resistant to ampicillin, while *S.* Blockley and *S.* Newport, had the highest percentage (50%) of isolates resistant to ciprofloxacin. Out of the resistant *S.* Enteritidis pulsotypes to ampicillin, JEGX01.0001 showed the highest resistance.

Table 2. Distribution of PFGE pulsotypes for *Salmonella* spp. isolated from clinical and food samples. PFGE: Pulsed-Field gel Electrophoresis.

Serotype	PFGE pattern	Total n (%)		Serotype	PFGE pattern	Total n (%)		Serotype	PFGE pattern	Total n (%)	
		Clinical	Food			Clinical	Food			Clinical	Food
***Salmonella* Typhimurium**	JPXX01.0001	1(0.3)	-	***Salmonella* Typhi**	JPPX01.0001	8(2.8)	1(2.0)	***Salmonella* Paratyphi A**	PA2	1(0.3)	-
	JPXX01.0002	5(1.7)	1(2.0)		JPPX01.0002	6(2.1)	-		PA3	-	1(2.0)
	JPXX01.0003	3(1.1)	-		JPPX01.0003	4(1.4)	-		PA4	-	1(2.0)
	JPXX01.0004	1(0.3)	3(6.1)		JPPX01.0004	1(0.3)	-		PA5	-	1(2.0)
	JPXX01.0005	16(5.5)	-		JPPX01.0005	-	1(2.0)		PA6	1(0.3)	1(2.0)
	JPXX01.0006	-	3(6.1)		JPPX01.0006	-	1(2.0)		PA7	-	1(2.0)
	JPXX01.0007	22(7.6)	2(4.1)	***Salmonella* London**	LO01	1(0.3)	-		PA8	-	1(2.0)
	JPXX01.0008	-	2(4.1)		LO02	1(0.3)	-		PA9	2(0.7)	1(2.0)
	JPXX01.0009	1(0.3)	-		LO03	2(0.7)	-		PA10	1(0.3)	-
	JPXX01.0010	-	1(2.0)		LO04	1(0.3)	-		PA11	1(0.3)	-
	JPXX01.0011	1(0.3)	1(2.0)		LO05	1(0.3)	-		PA12	1(0.3)	-
	JPXX01.0012	27(9.3)	-		LO06	1(0.3)	-		PA13	-	1(2.0)
	JPXX01.0013	1(0.3)	-		LO07	1(0.3)	-		Unsp.	1(0.3)	-
	JPXX01.0014	2(0.7)	-		LO08	1(0.3)	-	***Salmonella* Paratyhi C**	PTC1	2(0.7)	-
	JPXX01.0015	1(0.3)	-		Unsp.	1(0.3)	-	**Others**	-	13(4.5)	4(8.2)
	JPXX01.0016	1(0.3)	-	***Salmonella* Blockley**	BL01	1(0.3)	1(2.0)	**Total**		**290 (100)**	**49 (100)**
	Unsp.	2(0.7)	1(2.0)		BL02	1(0.3)	-				
***Salmonella* Enteritidis**	JEGX01.0001	110(40.0)	8(16.3)		BL03	-	1(2.0)				
	JEGX01.0003	-	1(2.0)		Unsp.	1(0.3)	-				
	JEGX01.0004	16(5.5)	-	***Salmonella* Newport**	JJPX01.0001	2(0.7)	-				
	Unsp,	-	1(2.0)		JJPX01.0002	-	1(2.0)				
***Salmonella* Braenderup**	JBPX01.0001	5(1.7)	-		JJPX01.0003	-	1(2.0)				
	JBPX01.0002	4(1.4)	-		JJPX01.0004	-	1(2.0)				
	JBPX01.0003	9(3.1)	4(8.2)		JJPX01.0005	-	1(2.0)				
	JBPX01.0004	1(0.3)	-	***Salmonella* Paratyphi B**	JXX01.0001	1(0.3)	-				
	JBPX01.0005	2(0.7)	-		Unsp.	1(0.3)	-				

Table 3. Distribution of resistance among *Salmonella* serotypes and PFGE types in the food samples**.** Percentage resistance of each serotype was calculated by identifying the number of resistant isolates in each serotype divided by the total number of isolates in that serotype. The percentage of resistance in each PFGE type was calculated by identifying the number of resistant isolates in each PFGE type divided by the total number of resistant isolates in each serotype. PFGE: Pulsed-Field gel Electrophoresis.

Salmonella **serotype- Clinical samples (n)**	**PFGE type**	**Ampicllin n (%)**	**Ceftazidime n (%)**	**Ciprofloxacin n (%)**	**Trimethoprim-sulfamethoxazole n (%)**
Typhimurium (84)	JPXX01.0001	1(3)	1(50)	-	-
	JPXX01.0002	5(15.2)	-	-	-
	JPXX01.0004	-	1(50)	-	-
	JPXX01.0005	2(6.1)	-	-	1(33.3)
	JPXX01.0007	6(18.2)	-	1(50)	1(33.3)
	JPXX01.0009	1(3)	-	1(50)	-
	JPXX01.0012	17(51.5)	-	-	-
	JPXX01.0015	1(3)	-	--	1(33.3)
	Total	**33(39.2)**	**2(2.4)**	**2(2.4)**	**3(3.6)**
***Enteritidis* (126)**	JEGX01.0001	15(88.2)	3(75)	2(100)	**-**
	JEGX01.0004	2(11.8)	1(25)	-	**-**
	Total	**17(13.5)**	**4 (3.2)**	**2 (1.6)**	**10 (8)**
Typhi *(*19)	JPPX01.0001	5(45.4)	1(100)	2(100)	5(62.5)
	JPPX01.0002	4(36.4)	-	-	3(37.5)
	JPPX01.0003	2(18.2)	-	-	-
	Total	**11(57.9)**	**1 (5.3)**	**2 (10.5)**	**8(42.1)**
Blockley	BL01	-	-	1(100)	-
	Total	**-**	**-**	**1(33.3)**	**-**
Braenderup	JBPX01.0005	1(100)	-	-	
	Total	**1 (4.8)**	**-**	**-**	**-**
Paratyphi A	PA2	1(50)	-	1(50)	-
	PA9	-	-	1(50)	-
	PA10	1(50)	-	-	-
	Total	**2(25)**	**-**	**2 (25)**	**-**
Paratyphi B	Unspec.	1(100)	-	1(100)	1(100)
	Total	**1(50)**	**-**	**1(50)**	**1 (50)**
Paratyphi C	-	-	-	-	-
London	Unspec.	-	-	-	1(100)
	Total	**-**	**-**	**-**	**1(10)**
Newport (2)	JJPX01.0001	1(100)	-	1(100)	-
	Total	**1(50)**	**-**	**1 (50)**	**-**
Others (13)	**Total**	**2(15.4)**	-	-	-
	Total resistance	**68**	**7**	**11**	**23**

Figure 2. Representative dendrograms of *Salmonella* spp. from clinical specimens matching those from food sample. F: Food isolates.

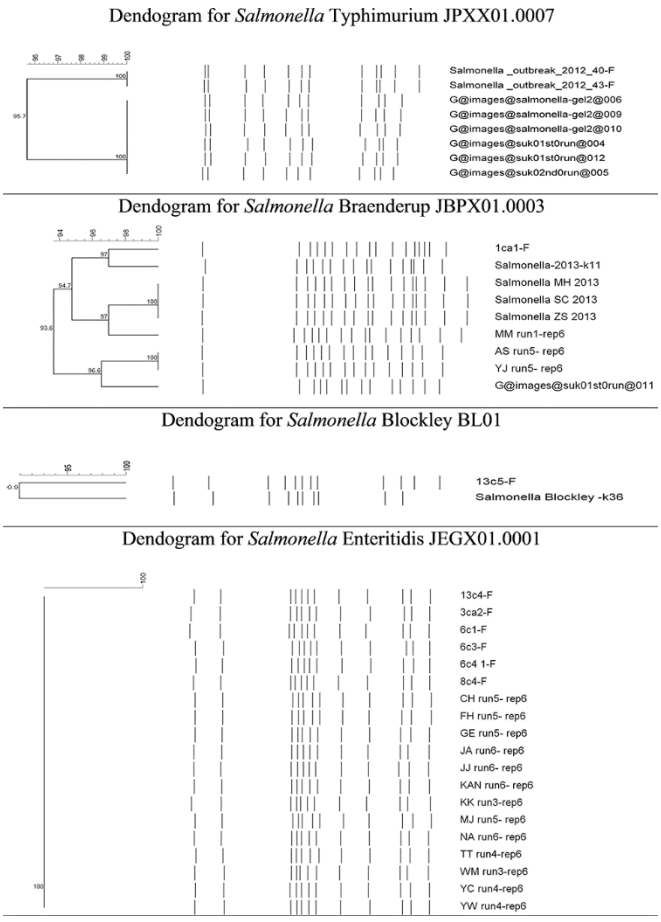

Figure 3. Percentage of antimicrobial resistance in *Salmonella* isolates from food samples and clinical specimens.

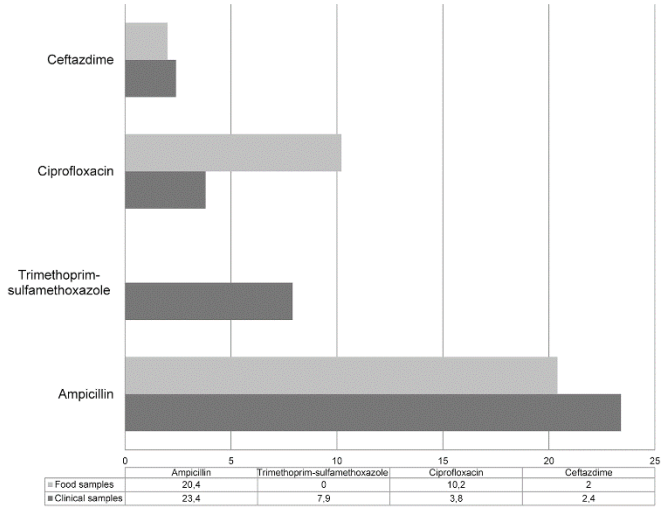

Table 4. Distribution of resistance among *Salmonella* serotypes and PFGE types in the clinical samples**.** Percentage resistance of each serotype was calculated by identifying the number of resistant isolates in each serotype divided by the total number of isolates in that serotype. The percentage of resistance in each PFGE type was calculated by identifying the number of resistant isolates in each PFGE type divided by the total number of resistant isolates in each serotype. PFGE: Pulsed-Field gel Electrophoresis.

***Salmonella* serotype- Food samples (n)**	**PFGE type**	**Ampicllin n (%)**	**Ceftazidime n (%)**	**Ciprofloxacin n (%)**	**Trimethoprim-sulfamethoxazole n (%)**
Typhimurium (14)	JPXX01.0002	1(100)	-	-	-
	Total	**1(7.1)**	**-**	**-**	**-**
Enteritidis (10)	JEGX01.0001	4(80)	-	1(100)	-
	Unspec.	1(20)	-	-	-
	Total	**5(50)**	**-**	**1 (10)**	**-**
Typhi (3)	JPPX01.0005	1(100)	-	1(100)	-
	Total	**1 (33.3)**	**-**	**1 (33.3)**	**-**
Blockley (2)	BL01	-	-	1(100)	-
	Total	**-**	**-**	**1 (50)**	**-**
Braenderup (4)	-	-	-	-	-
Paratyphi A (8)	PA3	1(100)	1(100)		-
	Total	**1 (12.5)**	**1 (12.5)**	**-**	**-**
Newport (4)	JJPX01.0003	1(50)	-	1(50)	-
	JJPX01.0005	1(50)	-	1(50)	-
	Total	**2 (50)**	**-**	**2 (50)**	**-**
Others (4)	-	-	-	-	-
	Total resistance	**10**	**1**	**5**	**0**

Regarding the *S.* Blockley pulsotypes, BL01 PFGE type had the highest percentage of resistance to ciprofloxacin. Out of the *S.* Newport resistant pulsotypes, the JJPX01.0003 and JJPX01.0005, exhibited the highest resistance to ampicillin and ciprofloxacin. The distribution of resistance among *Salmonella* serotypes and PFGE types in the clinical and food samples are shown in Figure 3, Table 3 and 4.

Discussion

This is the first study carried out in Lebanon to 1) identify the antimicrobial resistance profile, serotypes and PFGE pulsotypes of *Salmonella* in clinical and food samples and 2) to link sources to the clinical cases during outbreaks identified by the MoPH.

As a surveillance tool, serotyping of *Salmonella* has been used to characterize serotypes circulating in an area over a period of time and help in identification of outbreaks and their sources [16]. Our study showed that *S.* Enteritidis and *S.* Typhimurium were the two most common serotypes identified in clinical and food samples. Serotyping also showed the circulation of ten and seven serotypes in the clinical and food samples, respectively. Galanis *et al.* (2006) showed the distribution of *Salmonella* serotypes between 2000 and 2002 globally. The study revealed that in Arab countries such as Morocco and Tunisia, Asia (2002), and globally, *S.* Enteritidis and *S.* Typhimurium were the two most common serotypes isolated from humans. This is in accordance with our results. In non-human isolates, the most prevalent serotype globally was *S.* Typhimurium followed by *S.* Heidelberg and *S.* Enteritidis. However, both serotypes were not among the most commonly isolated serovars in non-human samples in Asia [17].

In 2012 and 2013, Araj *et al*. showed that the most common serotypes isolated from clinical samples in Lebanon at the AUB-MC were *S.* Enteritidis followed by *S.* Typhimurium [18,19]. *S.* Enteritidis, *S.* Typhimurium, *S.* Typhi, and *S.* Paratyphi A, B, and C were the common serotypes between our study and these studies. A reason why *S.* Enteritidis is usually the most commonly isolated serotype from clinical samples might be due to its presence in eggs and poultry products which are ingredients in a wide range of foods leading to major outbreaks [20]. Concerning animal related studies, a study carried out in Lebanon showed that *S.* Typhimurium, isolated from ten different animals, was the first and the second most common serotype of *Salmonella* to be isolated from chickens and cattle, respectively [21]. In United Arab Emirates, *S.* Typhimurium and *S.* Enteritidis were the first and fourth most frequently isolated serotypes from animals [22]. In line with this, our results showed that *S.* Typhimurium was the most frequently isolated serotype from food samples.

PFGE is one of the golden standard methods used to identify and epidemiologically differentiate *Salmonella* species during outbreaks. Our study showed a diverse banding pattern among most of the serotypes. However, *S.* Enteritidis, one of the most commonly isolated serotype in this study, had only 3 different pulsotypes in both food and clinical isolates. This might be due to its high genetic homogeneity [20]. Other serotypes such as *S.* Typhimurium, *S.* London, and *S.* Paratyphi A exhibited a large number of pulsotypes. Using PFGE, Saleh *et al* (2010) were able to detect three different outbreaks in the Northern Province of Lebanon and link them to their suspected food sources. They concluded that a single strain of *S.* Enteritidis, JEGX01.0001, has been circulating during that year [23]. This pulsotype has also been extensively isolated in our study in both clinical and food samples, indicating its continuing prevalence in Lebanon.

Antimicrobial resistance among pathogens is increasing due to: the excessive prescription of antimicrobial agents, the deliberate self-administration of antibiotics by patients themselves, and the indiscriminate usage of antimicrobial agents in animal farms. In our study, the highest resistance of *Salmonella* serovars in both clinical and food samples was to ampicillin. This might be as a result of the usage of broad spectrum antibiotics such as ampicillin for: the treatment of gastrointestinal diseases caused by contaminated food and water and the preservation of healthy livestock and rapid treatment of diseases on farms [24-27].

Several studies were conducted in Lebanon and the region concerning the resistance of *Salmonella* to antimicrobial agents in clinical cases and food samples. Clinical studies done by Araj *et al* at the American University of Beirut Medical Center (AUB-MC) between 1998- 2012 showed that: 1) the resistance of non-typhoidal *Salmonella* in clinical cases to ampicillin was moderate and that to trimethoprim-sulfamethoxazole and a third generation cephalosporin, cefotaxime, was low during 1998 and 2000 [24, 28]; 2) prior to 2004, *S.* Typhi was susceptible to antimicrobial agents, after which it started showing resistance to ampicillin and trimethoprim-sulfamethoxazole [18]. These studies are in accordance to our study in which 1) *Salmonella* serovars showed a high resistance to ampicillin and low resistance to trimethoprim-sulfamethoxazole and ceftazidime, a third generation

cephalosporin; 2) *S*. Typhi, between 2011 and 2014, had one of the highest percentages of serovars showing resistance to antimicrobial agents. Concerning food related studies, a study done by Harakeh *et al*. (2006) showed that *Salmonella* serovars from meat based fast foods exhibited a very high resistance to trimethoprim-sulfamethoxazole and a moderate resistance to cefotaxime [29]. Similarly, a study done in the United Arab Emirates for a period of 14 years concerning antimicrobial susceptibilities of *Salmonella* serovars in animals revealed that the resistance to ampicillin and ciprofloxacin has been declining in contrast to trimethoprim-sulfamethoxazole to which the resistance has been rising [22]. Our study, on the contrary, showed that: *Salmonella* in food samples had the highest resistance to ampicillin followed by ciprofloxacin and all the isolates were susceptible to trimethoprim-sulfamethoxazole.

Conclusion

This study emphasizes the importance of surveillance through a "farm-to-fork" approach in detecting and identifying widely circulating food borne pathogens in Lebanon such as *Salmonella*, that can cause outbreaks. This will in due course guide the MoPH in taking the right preventative measures for food-borne diseases. Additionally, the knowledge obtained about the antimicrobial resistance patterns from clinical isolates will guide physicians in selecting appropriate treatment regimens. Food-borne illnesses remain a main endemic health issue, even though Lebanon has undertaken several measures in food safety. This calls for setting out and implementing regulations on food available in the market and emphasizes the need to improve methods in detecting and investigating disseminated outbreaks. Additionally, even though food contamination can take place at any phase of the food production, improper preparation or handling of food at homes or food establishments and the lack of proper food hygiene can cause foodborne diseases. Therefore, education of food handlers and implementation of basic food hygiene is important in the prevention of such diseases.

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