SEROTYPING AND ANTIMICROBIAL RESISTANCE OF SALMONELLA ISOLATES FROM FOOD MATRICES AND CLINICAL SPECIMENS FROM LEBANON

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ABSTRACT

Salmonella is a genus of bacteria responsible for the most frequently reported cases of foodborne illnesses worldwide. In this study, serotyping was done by using highly specific manufactured antisera, to identify circulating *Salmonella* serovars isolated from food samples and clinical specimens. Antibiotic susceptibility testing was also performed to determine the level of resistance of these serotypes. This was done by using the disk diffusion method by testing 14 clinically important antibiotics on the *Salmonella* isolates. A total of 85 *Salmonella* strains, preidentified by biochemical testing, were collected over the course of one year. Serotyping of the isolates revealed the presence of 14 serotypes in the food samples with *S.* Infantis and *S.* Enteritidis, being the most common and 6 serotypes in the clinical samples with *S.* Enteritidis and *S.* Typhimurium being the most common. Antimicrobial susceptibility testing results showed resistance of isolates from both sample types to conventional antibiotics like ampicillin and piperacillin, and almost total resistance of the food isolates to a broad-spectrum antibiotic imipenem, belonging to the carbapenem class. Higher resistance was observed in food samples than in clinical ones. Moreover, 13 and 3 multidrug resistant serotypes of *Salmonella* were identified for the first time in Lebanon in the food and clinical samples respectively, with three of them isolated from imported food samples.

Keywords: Salmonellosis, Multidrug resistant bacteria, Foodborne diseases, Pathogenesis

INTRODUCTION

Foodborne bacterial diseases constitute a growing challenge to human health around the world, in both developing and industrialized countries **(Sánchez-vargas *et al.,* 2011)**. Case reports of food intoxications and infections caused by these pathogens are increasing as a consequence of changes in the natural environment, human lifestyle and the haphazard use and prescription of antibiotics, causing bacterial resistance and outbreaks **(Scallan *et al.,* 2011)**. Several pathogens like *Salmonella*, *Escherichia coli, Yersinia enterolitica, Staphylococcus aureus*, *Clostridium botulinum* and many more, are causing foodborne outbreaks and becoming economic burdens on many societies, as they associate with new types of food vehicles and develop resistance to common antibiotics **(Masterton, 2008; Pires *et al.,* 2014)**. Of these pathogens, the genus *Salmonella* and specifically non-typhoidal *Salmonella* rank second after Norovirus in the top five pathogens contributing to domestically acquired foodborne illnesses published by the CDC **(Scallan *et al.,* 2011)**.
The *Salmonella* genus encompasses many strains and serotypes that cause an array of diseases, ranging from mild gastroenteritis to life threatening major diseases like typhoid fever, caused by *Salmonella* Typhi and Paratyphi serotypes, especially in the poorer regions of the world and developing countries like Lebanon **(Ryan *et al.,* 2017)**. This genus contains more than 2,500 serotypes, at present, and has a complex nomenclature **(Sloan *et al.,* 2017)**. Hence, serotype identification is of great importance in order to monitor and contain outbreaks associated with salmonellosis cases, and link them with their respective contaminated food sources for the purpose of prioritizing effective food safety interventions. Continuous updates of the identification and tracking of new serovars is of utmost importance for the long term epidemiological surveillance of this ubiquitous pathogen found in the food chain, in order to establish control in the public health sector in Lebanon **(Malaeb *et al.,* 2016).**
Add to that, antibiotic resistance is actually reaching dangerously high levels in the world. New resistance mechanisms are emerging and spreading across the globe, compromising our ability to treat common infectious diseases. For an increasing number of infections, such as tuberculosis, sepsis, pneumonia, gonorrhea and foodborne diseases, treatment becomes more difficult, if not sometimes impossible, due to the loss of effectiveness of antibiotics **(World Health Organization, fact sheet).**
The objective of our study was to identify, by serotyping, the circulating and predominant serovars of *Salmonella* isolated from clinical and food sources in Lebanon, and to establish the current antibiotic resistance patterns of the identified serotypes of *Salmonella*

MATERIALS AND METHODS

Bacterial isolates
Clinical isolates

Over the period of one year (October 2018 - October 2019), 16 clinical isolates already identified as *Salmonella* . with API 20E, were collected from different hospitals, pertaining from the stools of patients with salmonellosis.

Food isolates

Also during the period from October 2018 to October 2019, a total of 69 strains of *Salmonella* were collected from different food matrices and identified with API 20E. They were provided by the Lebanese Agricultural Research Institute (LARI) and the Chamber of Commerce, Industry & Agriculture at Tripoli & North Lebanon (CCIAT).

Serotyping

Serotyping was performed by following guides and protocols published by Bio-Rad. (n.d.-b). The detailed flowchart of *Salmonella* serotyping is represented in figure 1.
The isolated *Salmonella* strains were streaked onto a solid Trypticase Soya Agar (TSA) (VWR International Eurolab, Barcelona, Spain), a non-selective agar medium, to obtain pure colonies. Cultures were incubated at 37°C for 18 hours.

Autoagglutination Test

The auto-agglutination test was performed by mixing pure colonies with one drop of physiological water/saline (0.85%). If auto-agglutination was observed, the strain was recorded as being unable to be serotyped.

Testing with Omni-O antiserum

All strains were tested with *Salmonella* Omni-O antiserum for the presumptive identification of O-agglutinable strains of *Salmonella*. If agglutination exists, the strain is O-agglutinable and can be further serotyped with specific antisera.

Testing for O Antigens

The tests for O Antigens (polyvalent O sera, monovalent O sera and Anti-Vi serum) and for H antigen phase I and phase II (Polyvalent H sera and monovalent H sera) were done according to the bacterial serotyping guide for *Salmonella* **(Bio-Rad, FSD_14-0699.pdf)**.

Phase inversion – Sven Gard method

After identification of the first phase, the Sven Gard agar medium [supplied in tubes, 25 mL, Bio-Rad (Marnes-la-Coquette, France)] was melted in a water bath of 95°C. After Cooling to 44°C, one drop of SG sera, containing the agglutinin of the phase already determined, was added. Tubes were then mixed with circular motions, to ensure homogenization of the serum before pouring onto Petri-dishes. After solidification, 3 to 4 colonies from a pure culture of *Salmonella* were inoculated at the center of the plate and incubated, with the dish cover facing upwards, for 18 hours at 37°C. The H phase II antigen was then identified by slide agglutination, by collecting the culture from the periphery of the invasion zone.
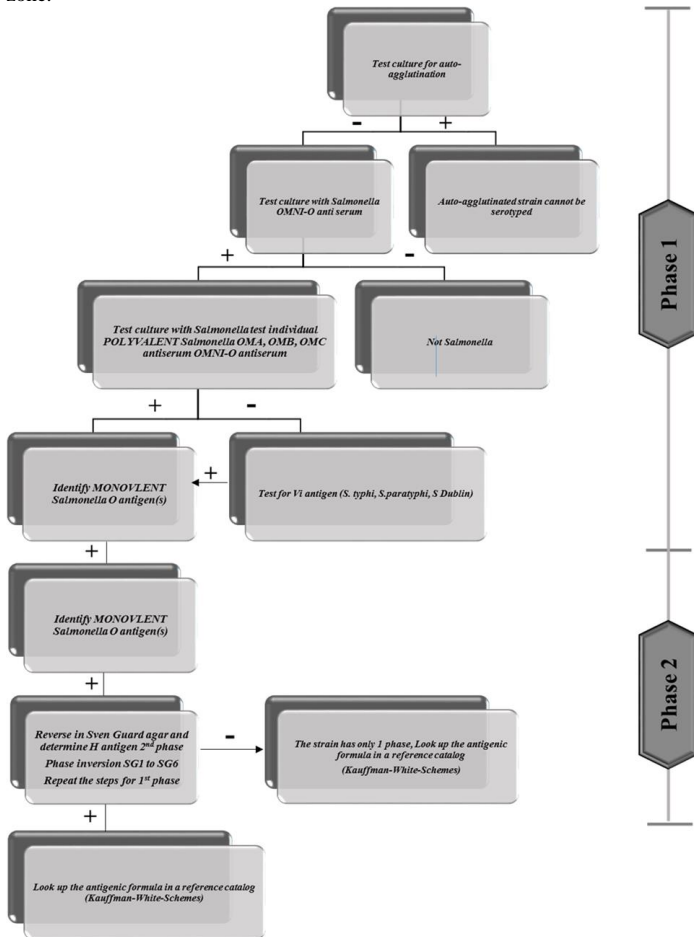

Figure 1 Detailed flowchart of *Salmonella* spp. serotyping (Bio-RAD, France)

Identification and the Antigenic formula

After the identification of the O, H-phase I and H-phase II antigens, they were placed in the antigenic formula to identify the serotype. This was done by referring to the White-Kauffman-Le Minor scheme **(Grimont *et al.,* 2007)**. The formula is as follows: O antigens in Arabic numeral, Vi (when present), H antigens phase I in lower case letters, H antigens phase II in Arabic numerals (when present).

The serotyping steps described previously, are summarized in the flowchart represented in figure 1.

Antibiotic susceptibility testing

Antibiotic resistance testing was performed on the serotyped strains of *Salmonella (spp).* by using the disk diffusion method (Kirby Bauer method) according to the guidelines of the Clinical & Laboratory Standards Institute (CLSI) **(Performance Standards for Antimicrobial Susceptibility Testing, 2017).** The susceptibility testing was done using 14 antibiotics (total of seven classes) commonly used for the treatment of infections caused by Enterobacteriaceae (Table 1). Antibiotic disks were purchased from Sigma Aldrich (Darmstadt, Germany). These disks were placed on the surface of an agar plate previously inoculated with the bacterial strain and plates were incubated at 37 ˚C for 18 hours. After the incubation period, zones of inhibition were recorded using a ruler to the nearest millimeter and classified as resistant (R), intermediate (I) or susceptible (S) based on the guidelines of the Clinical & Laboratory Standards Institute (CLSI) **(Performance Standards for Antimicrobial Susceptibility Testing, 2017).**

Table 1 List of Antibiotics used for the study

Antibiotics	Family	Abbreviations
Amoxicillin-clavulanic acid (20/10 µg)	β Lactam / β Lactamase inhibitor	AMC
Amikacin (30 µg)	Aminoglycosides	AK
Ampicillin (10 µg)	Penicillins	AMP
Aztreonam 30 µg	Monobactams	ATM
Ciprofloxacin (5 µg)	Fluoroquinones	CIP
Cefamandole (30 µg)	Cephalosporins	MA
Ceftriaxone (30 µg)	Third generation Cephalosporins	CRO
Imipenem (10 µg)	Carbapenems	IMI
Gentamycin (10 µg)	Aminoglycosides	CN
Ofloxacin (5 µg)	Fluoroquinones	OFX
Piperacillin (100 µg)	Penicillins	PRL
Piperacillin - Tazobactam (100/10 µg)	β Lactam / β Lactamase inhibitor	TZP
Tobramycin (10 µg)	Aminoglycosides	TOB
Trimethoprim Sulfamethoxazole (1.25/ 23.75 µg)	Folate pathway inhibitors	SXT

RESULTS AND DISCUSSION

Serotyping

Serotyping of the clinical samples showed a high prevalence of *S.* Enteritidis (56.25%) being the most commonly isolated serovar, Followed by *S.* Typhimurium (12.50%) being the second most isolated one (Figure 2). However, the most prevalent serotype in the food samples was *S.* Infantis (24.63%) followed by *S.* Enteritidis (18.84%) (Figure 3).
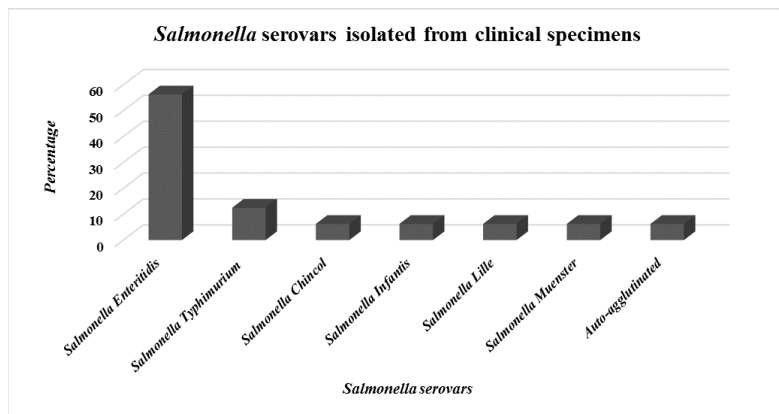

Figure 2 Percentage of *Salmonella* serovars isolated from clinical specimens
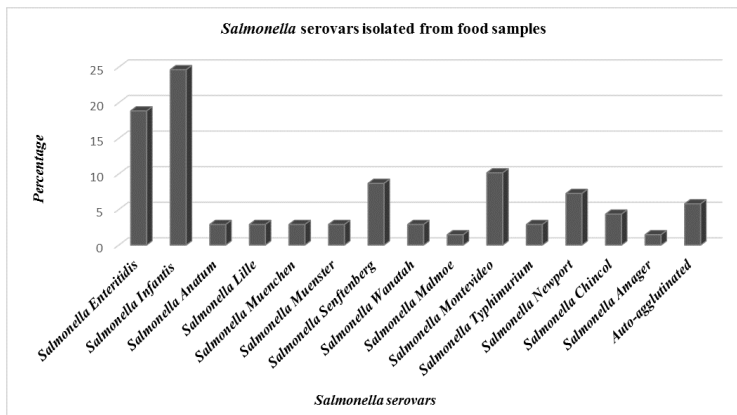
Figure 3 Percentage of *Salmonella* serovars isolated from food samples

Food samples showed more variety in serotypes like *S.* Montevideo, *S.* Senftenberg, *S.* Newport, *S.* Anatum, *S.* Muenchen, *S.* Wanatah (Figure 3). Sources Data of all *Salmonella* serovars from local and imported food matrices are shown in table 2. Chicken followed by beef, Tahina and sausages, were shown to be the most prevalent food sources of isolated *Salmonella* serovars.

Of all the food origins, three food types are widely consumed by the Lebanese population as traditional cuisine: chicken Tawouk, raw meat and minced meat. *S.* Enteritidis was mostly isolated from chicken "Tawouk"; a traditional Middle Eastern food consisting of chicken cubes marinated in tomato paste and different spices, and minced meat. As for *S.* Infantis, this serovar was isolated from chicken dishes that can contain eggs in their breading, and ham in their filling; hence, source attribution for *S.* Infantis can be uncertain since this serotype can be isolated from chicken, eggs and swine. All the other serotypes in our study are mainly associated with their common reservoirs. The antigenic formula of the identified *Salmonella* serovars from clinical and food samples are represented in table 3.

Table 2 Food sources of the serotypes *Salmonella* serovars from local and imported food items

Serotypes	Food sources
Salmonella Enteritidis	Escalope (chicken)
	Tawouk chicken
	Minced meat
	Chicken breast
Salmonella Infantis	Chicken feet
	Chicken nuggets
	Cordon bleu chicken
	Chicken slices
	Tawouk
Salmonella Muenster	Minced meat
	Raw meat
	Tahina
Salmonella Senftenberg	Tahina
	Frozen beef
Salmonella Lille	Broiler chicken
	Chicken breast
Salmonella Anatum	Soujouk (sausage)
	Hamburger (beef)
Salmonella Wanatah	Frozen chicken breast
Salmonella Muenchen	Frozen chicken breast imported from Brazil
Salmonella Typhimurium	Minced meat
Salmonella Montevideo	Sausage
Salmonella Malmoe	Frozen beef imported from India
Other	Frozen chicken breast
	Raw meat
	Soujouk (Sausage)

The serotyping results of the clinical samples of this study are in agreement with the study of **Fadlallah *et al.* (2017),** who performed the serotyping of *Salmonella* spp. isolates, collected between 2011 and 2014 in Lebanon. Their study showed the prevalence of S. *Enteritidis* among both human and food samples, with *S.* Typhimurium being the second prevalent serotype in both types of samples.

However, in our study, the first most prevalent serotype in the food samples was S. Infantis (24.63%) and S. Enteritidis (18.84%) had a lower prevalence, but still being the most commonly isolated serotype when added to the clinical serovars in term of percentages. The shift in prevalence from S. Typhimurium to S. Infantis is noted in many recent studies. The main reason can be explained by the emergence of new S. Infantis clones that have enhanced capabilities to colonize food animals, and spread rapidly (**kalaba et al., 2017; Antunes et al., 2016).**

Table 3 The antigenic formula of Salmonella serovars from clinical and food samples

Nomenclature	OMNI-O	Antigenic Formula
Salmonella Enteritidis	+	1,9,12 : g,m : -
Salmonella Infantis	+	6,7,14 : r : 1,5
Salmonella Montevideo	+	6,7,14 : g,m,[p],s : [1,2,7]
Salmonella Senftenberg	+	1,3,19 : g,[s],t : -
Salmonella Newport	+	6,8,20 : h : 1,2
Salmonella Typhimurium	+	1,4,[5],12 : i : 1,2
Salmonella Chincol	+	6,8 : g,m,[s] : [e,n,x]
Salmonella Lille	+	6,7,14 : z38 : -
Salmonella Amager	+	3,10,15 : y: 1,2
Salmonella Malmoe	+	6,8 : i : 1,7
Salmonella Wanatah	+	1,3,19 : d : 1,7
Salmonella Muenchen	+	6,8 : d : 1,2
Salmonella Muenster	+	3,{10}{15}{15,34} : e,h : 1,5
Salmonella Anatum	+	3,10,15 : h : 1,6

Food samples showed more variety in serotypes, with most of them not identified in the clinical samples. All the serotypes isolated from food samples, excluding *S.* Wanatah, do cause illness in humans, however, a reason for not finding them in our clinical samples might be due to the difference in sampling sizes, with food isolates (n=69) being more numerous than the clinical samples (n=16). The five strains that were not serotyped and were labeled as "Auto-agglutinated" (for both clinical and food samples).

By comparing the serotypes that were found in our study, and the ones identified by **Fadlallah *et al.* (2017)**, we notice the absence of most serotypes like *S.* Typhi, *S.* Braenderup, *S.* Blockley and *S.* Paratyphi A in our food samples. However, lack of food source data, along with the country or region of origin, in their study, makes it difficult to compare to our samples which contained serotypes that are less commonly isolated like *S.* Malmoe and *S.* Muenster, with the latter being an invasive serotype that can contribute to an increase in fatality rates (**Andino *et al.,* 2015).** Valuable information was deduced from the food sources of the isolates, poultry constituted the main food reservoir of the totality of food sources. This is in accordance with many studies that confirm poultry as the main reservoir responsible for *Salmonella* spp. outbreaks (**Andino *et al.,* 2015).** Furthermore, the countries of origin of the foods also revealed that four imported food items found in this study contained three different serovars *S.* Senftenberg - India, *S.* Malmoe - India, and *S.* Muenchen – Brazil, with the two latter being uncommon serotypes.

Antibiotic susceptibility testing

Clinical strain

From the 16 clinical isolates, 13 were resistant to at least one antimicrobial agent from 14 tested antibiotic agents. All the isolates were susceptible to aztreonam (ATM). Almost total susceptibility was also observed for ciprofloxacin and ofloxacin (OFX) (93.75% each). In addition, 87.5 % of the isolates were susceptible to amoxicilin – clavulanic acid (AMC) and 81.25% of the isolates in this study were susceptible to both trimethoprim - sulfamethoxazole (SXT) and gentamycin (CN). Moreover, 6.25% of the isolates were susceptible to piperacillin and 25 % were susceptible to ampicillin; both belonging to the penicillin antibiotic class. However, the use of piperacillin with a β lactamase inhibitor like tazobactam (TZP) increased the percentage of the susceptible isolates (75 %). Of particular note, none of the isolates was susceptible to imipenem (IMI) (Table 4).

The antimicrobial resistance profile of the clinical isolates is shown in table 5. Results showed that three out of the 16 strains (18.75%) exhibited multidrug resistance profile (MDR), defined as resistance to at least three different families of antimicrobials. *S.* Lille displayed resistance to 5 antibiotic families and 7 antimicrobial agents, and was intermediate for a 6th antibiotic class also. *S.* chincol and the autoagglutinated strain displayed resistance to 6 and 5 antimicrobial agents respectively (Table 5).

Table 4 Percentage of Susceptible (S), Intermediate (I) or Resistant (R) serotypes isolated from clinical samples (n=16) for 14 antibiotics

Antibiotics	Percentage (S, I, R) for each antibiotic		
	S%	I%	R%
AMC	87.5	0	12.5
AK	75	25	0
AMP	25	37.5	37.5
ATM	100	0	0
CIP	93.75	6.25	0
MA	87.5	6.25	6.25
CRO	62.5	18.75	18.75
IMI	0	62.5	37.5
CN	81.25	12.5	6.25
OFX	93.75	6.25	0
PRL	6.25	31.25	62.5
TZP	75	12.5	12.5
TOB	87.5	6.25	6.25
SXT	81.25	6.25	12.5

Table 5 Antimicrobial resistance phenotypes of Salmonella from clinical sources and the presence of multidrug resistance (MDR) strains

Resistance phenotypes	Serotype (number of strain)	MDR
IMI	*S.* Enteritidis *(n=2)*	-
PRL	*S.* Enteritidis *(n=2)* *S.* Typhimurium *(n=1)* *S.* Infantis *(n=1)*	-
AMP-PRL	*S.* Enteritidis *(n=1)*	-
CRO-CN	*S.* Enteritidis *(n=1)*	-
AMC-AMP-PRL	*S.* Typhimurium *(n=1)*	-
AMP-IMI-PRL	*S.* Enteritidis *(n=1)*	-
AMC-AMP-CRO-IMI-PRL	Autoagglutinated *(n=1)*	+
AMP-MA-IMI-PRL-TZP-SXT	*S.* Chincol *(n=1)*	+
AMP-CRO-IMI-PRL-TZP-TOB-SXT	*S.* Lille *(n=1)*	+

Food strains

Results of antimicrobial susceptibility testing of *Salmonella* isolates from food are summarized in Table 6. Results showed that most of the isolates were susceptible to aztreonam (ATM) (95.31%), ceftriaxone (87.5%), cefamandole (MA) (85.94%), trimethoprim sulfamethoxazole (SXT) (85.94%) and amoxicillin clavulanic acid (AMC) (71.88%). Strains were commonly resistant to imipenem (IMI) (79.69 %) and showed resistance to ampicillin (50%) and piperacillin (25%). As for the clinical isolates, none of the food isolates was susceptible to imipenem (Table 6).
The table 7 show the antimicrobial resistance profile of food isolates where 58 among the 69 tested isolates, show a resistance to at least one family of the tested antibiotics. Among these isolates, 13 showed MDR profile (Table 7). MDR isolates were *S.* Senftenberg (n=2), *S.* Anatum (n=1), *S.* Newport (n=1), *S.* Muenster (n=1), *S.* Montevideo (n=1), *S.* Chincol (n=2), *S.* Infantis (n=1), Autoagglutinated (n= 1), *S.* Malmoe (n=1) and *S.* Muenchen (n=2) (Table 7).

Table 6 Percentage of Susceptible (S), Intermediate (I) or Resistant (R) serotypes isolated from food samples (n=64) for 14 antibiotics

Antibiotics	Percentage (S, I, R) for each antibiotic		
	S (%)	I (%)	R (%)
AMC	71.87	12.5	15.63
AK	60.93	28.13	10.94
AMP	20.31	29.69	50
ATM	95.32	0	4.68
CIP	73.44	25	1.56
MA	85.94	7.81	6.25
CRO	87.5	7.81	4.69
IMI	0	20.31	79.69
CN	73.43	15.63	10.93
OFX	78.13	15.62	6.25
PRL	20.31	54.69	25
TZP	34.37	48.44	17.19
TOB	76.56	21.88	1.56
SXT	85.94	10.94	3.12

Table 7 Antimicrobial resistance phenotypes of Salmonella serovars from food sources and the presence of multidrug resistance (MDR) strains

Resistance phenotypes	Serotype (number of strain)	MDR
AMP	Autoagglutinated (1)	-
IMI	*S.* Infantis *(n=7)* *S.* Enteritidis *(n=8)* *S.* Montevideo *(n=2)* *S.* Senftenberg *(n=2)* *S.* Newport *(n=1)* *S.* Chincol *(n=1)* *S.* Anatum *(n=1)* *S.* Muenster *(n=1)*	-
CN	*S.* Montevideo *(n=1)*	-
AMC-IMI	*S.* Typhimurium *(n=1)*	-
AK-IMI	*S.* Infantis *(n=1)*	-
AMP-IMI	*S.* Infantis *(n=6)* *S.* Enteritidis *(n=3)* *S.* Montevideo *(n=1)* *S.* Newport *(n=1)* *S.* Lille *(n=1)* Autoagglutinated *(n=1)*	-
IMI-CN	*S.* Montevideo *(n=1)*	-
AMC-AMP-PRL	*S.* Wanatah *(n=1)*	-
AK-AMP-IMI	*S.* Senftenberg *(n=1)*	+
AK-IMI-PRL	*S.* Anatum *(n=1)*	+
AMP-IMI-PRL	*S.* Enteritidis *(n=1)* *S.* Montevideo *(n=1)*	-
AMC-AMP-IMI-PRL	*S.* Lille *(n=1)*	-
AK-AMP-IMI-TZP	*S.* Newport *(n=1)*	+
AK-IMI-PRL-TZP	*S.* Muenster *(n=1)*	+
AMC-AMP-IMI-CN-OFX	*S.* Montevideo *(n=1)*	+
AMP-IMI-OFX-PRL-TZP	*S.* Chincol *(n=1)*	+
AMP-IM-OFX-TZP-SXT	*S.* Chincol *(n=1)*	+
AMP-IMI-PRL-TZP-SXT	*S.* Infantis *(n=1)*	+
AK-IMI-CN-PRL-TZP-TOB	*S.* Senftenberg *(n=1)*	+
AMC-AMP-ATM-MA-CRO-IMI-PRL-TZP	Autoagglutinated *(n=1)*	+
AMC-AMP-CIP-MA-IMI-CN-OFX-PRL-TZP	*S.* Malmoe *(n=1)*	+
AMC-AMP-ATM-MA-CRO-IMI-CN-PRL-TZP	*S.* Muenchen *(n=1)*	+
AMC-AK-ATM-CIP-MA-CRO-IMI-CN-PRL-TZP	*S.* Muenchen *(n=1)*	+

The novelty of this study is the use of 14 clinically important antibiotics used for the treatment of salmonellosis in Lebanon. Other studies have performed antibiotic susceptibility testing on *Salmonella*, but their focus was based on a limited number of antibiotics like penicillins, fluoroquinolones and cephalosporins (**Jorgensen *et al.,* 2009; Percival *et al.,* 2014**). It is very important to monitor resistance to all types of antibiotics, in order to track the extent and prevalence of resistance in the different serotypes of *Salmonella* (**Percival *et al.,* 2014**). The increase of antimicrobial - resistant *Salmonella* strains is a global concern, it stems from the abusive use of antibiotics in humans and in farm animals for the mass production of food and animal byproducts (**Kolár *et al.,* 2001**).
Results of this study have shown that for both the clinical and food serovars, low susceptibility was observed for penicillins that include piperacillin and ampicillin, on the other hand, low resistance to almost none was observed for trimethoprim-sulfamethoxazole. Low susceptibility to ampicillin and low resistance to trimethoprim-sulfamethoxazole was also the case with the isolates of (**Fadlallah *et al.,* 2017**). Moreover, other studies also indicate that conventional antibiotics; used as first-line treatment, like ampicillin are no longer an appropriate choice for the treatment of invasive salmonellosis (**Su *et al.,* 2004**).
In addition, the results also indicate that none of the isolates were susceptible to imipenem in both sample types. This finding of carbapenem resistance is alarming, since this broad spectrum antibiotic is classified as a "critically important" and is often used as a "last resort agent", when patients with the infection become gravely ill or harbor resistant bacteria (**Papp-Wallace *et al.,* 2011**). Testing with other antibiotics from the carbapenem family, phenotypic and molecular studies might help to identify the cause of this non-susceptibility to imipenem; it might be due to the presence of transferred plasmids containing genes encoding resistance to carbapenems, via intra- and inter-species transfer by horizontal dissemination during conjugation (**EFSA, 2008**). The most striking results in this study was the presence of MDR strains in both sample types. These were resistant to the clinically important antibiotics. What is more concerning is that food samples contained more MDR strains (13 MDR strains) than the clinical samples (three MDR strains), furthermore, the 13 MDR

serotypes included the serotypes *S.* Muenchen *, S.* Malmoe and *S.* Senftenberg with the first two serotypes found in three imported food samples. According to the CDC, *S.* Muenchen is a commonly isolated MDR strain from South America (Brazil), and *S.* Senftenberg is considered a serotype that has higher resistance to heat up to 70 ˚C (**Manas *et al.,* 2003**). It has a thermotolerance approximately 30 times more than *S.* Typhimurium, hence surviving high cooking temperatures and transmission to humans.

By importing food, a variety of uncommon and MDR serovars of *Salmonella* are being introduced into the country, these strains have increased virulence and a high potential for survival and spreading to humans through the food chain, hence increasing the dissemination of more virulent serotypes of *Salmonella* in Lebanon and creating possible endemic outbreak situations.

Infection with MDR *Salmonella* strains would result in an increased number of treatment failures, since the normally used antibiotics are no longer effective. Physicians later would have to use "last resort" antibiotics that are often more expensive and toxic (**Kolár *et al.,* 2001; Papp-Wallace *et al.,* 2011; EFSA, 2008**).

CONCLUSION

Globalization of food-animal production is currently causing a major impact in antimicrobial resistance of zoonotic bacteria like *Salmonella* (**Hur *et al.,* 2012; Silbergeld *et al.,* 2008**). Selective pressure caused by the usage of clinically important antibiotics in feed animals to avoid animal sickness or as growth promoters, is leading to a rise in MDR isolates in animals and consequent dissemination of these strains in all the food chain (**Kolár *et al.,* 2001; Angulo *et al.,* 2004**). This can be an explanation to why the mean percent resistance in food samples was higher than the one for clinical samples in this study. These resistant bacteria are reaching humans by food consumption and causing major infections with a limited number of antibiotics to cure them. This is mostly observed in poultry production, which is one of the most consumed and increasing globally traded meat products, hence, it is crucial to restrict global usage of antibiotics in veterinary and farm settings to minimize the selection of resistant *Salmonella* (**Hur *et al.,* 2012; Kalaba *et al.,* 2017**).

Antibiotic susceptibility testing is an important tool to keep track of antibiotic resistance levels in various microorganisms, in different countries and throughout the world. This surveillance tool enables us to notice that resistance to antibiotics has risen greatly and quickly over the years, it also helps to identify MDR pathogens capable of fast dissemination (**Jorgensen *et al.,* 2009; Percival *et al.,* 2014**). Such knowledge allows us to focus on the development of new and improved antibiotics and alternative strategies to manage and prevent the spread of infectious foodborne diseases (**Kerr *et al.,* 2005**).

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