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A national study through a 'Farm-to-fork' Approach to determine *Salmonella* dissemination along with the Lebanese poultry production chain

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Abstract

This cross-sectional study was conducted to determine the prevalence of *Salmonella* at different stages of the broiler production chain and layer flocks in addition to their antibiotic resistance profile and molecular patterns. Over a period of 3 years, different sample matrices were collected from Lebanese farms, slaughterhouses and retail markets. Out of 672 *Salmonella* serotyped, 514 were analysed for antimicrobial resistance and 214 for clonality using Pulsed-field gel electrophoresis (PFGE). The results highlighted an important prevalence of *Salmonella*, 30% in farms, 35.8% in slaughterhouses and 22.4% at retail level. A large diversity of serotypes was identified with predominance among *Salmonella* Infantis (32.9%), *Salmonella* Enteritidis (28.4%) and *Salmonella* Kentucky (21.4%). High resistance to nalidixic acid was revealed in all the isolates. The most prominent resistance was exhibited in *S*. Kentucky and *S*. Infantis. The latter was resistant to tetracycline (99%), streptomycin (88.2%) and remarkable multi-drug resistance (MDR) (89.7%). All *S*. Kentucky isolates were resistant to ciprofloxacin, MDR (62.4%) and 6% were resistant to extended-spectrum cephalosporin (ESCs). One persistent clone of *S*. Enteritidis was found common between poultry and humans. Similar genomic profiles were detected between farms, slaughterhouses and retail suggesting the dissemination of identical clones throughout the food chain possibly due to weak barriers preventing such transmission.

KEYWORDS
antimicrobial resistance, poultry chain, prevalence, pulse-field gel electrophoresis, *Salmonella* spp

1 | INTRODUCTION

Foodborne *Salmonella* continues to be a major threat for public health (European Food Safety Authority/European Centre for Disease Prevention & Control, 2017). It is estimated that non-typhoidal *Salmonella* causes 93.8 million cases of gastroenteritis and 155,000 annual deaths worldwide (Majowicz et al., 2010). Poultry are the primary source of human infection triggered by the consumption of contaminated poultry products, such as meats and eggs (Foley et al., 2011). Although *Salmonella enterica* subspecies *enterica* cover more than 2,500 serotypes, only few are isolated from poultry with *S*. Enteritidis and *S*. Typhimurium being the predominant contaminant implicated in human gastroenteritis (EFSA/ECDC, 2017; Ricke et al., 2018). Other poultry-associated serotypes have emerged, including *S*. Infantis, *S*. Kentucky and *S*. Heidelberg, with this emergence particularly tormenting since these serotypes are frequently resistant to antibiotics (Gieraltowski et al., 2016; Le Hello et al., 2013; Nógrády et al., 2007).

In fact, in recent years, an increasing trend of antimicrobial resistance (AMR) was noticed, causing 25,000 annual deaths in Europe, 100,000 in USA and 80,000 in China (Ferri et al., 2017). MDR *Salmonella* strains and extended-spectrum-β-lactamase (ESBL)-producing serotypes are also increasing and constitute an emerging public health concern (Franco et al., 2015; Wasyl & Hoszowski, 2012). Despite being a self-limited infection, the elderly, infants and immunosuppressed might need antimicrobial therapy to treat salmonellosis. Some of these drugs such as fluoroquinolones and extended-spectrum cephalosporin are critically important for human medicine (Medalla et al., 2017), but their effectiveness is questionable and worrisome. This serious public health risk is mainly attributed to the inappropriate use (therapeutic, preventive and growth promoter) of antimicrobials in the animal sector (Ferri et al., 2017).

In Lebanon, the poultry sector has experienced rigorous growth where 80 million broilers are produced annually and is dominated by 10 large-scale slaughterhouses and poultry farms; four of them control more than half of the Lebanese market. Despite the poultry sector development, and the eminent risk of salmonellosis of poultry origin, information on *Salmonella* dissemination along the chain were either unavailable or incomplete.

The objective of this study was to determine *Salmonella* prevalence within a farm to fork approach, starting from broiler breeder farms to slaughterhouses and the retail (supermarkets and restaurants) and layer flocks. Serotypes circulation, antibiotic resistance and their genotypic relatedness were studied. Moreover, this work will serve as a database for a national strategy, surveillance programs and intervention measures, set by local authorities (Ministry of Agriculture) for prevention and control of salmonellosis in human and *Salmonella* dissemination in the poultry industry.

Impacts

- It is the first study in Lebanon determining the prevalence and antimicrobial resistance of *Salmonella* in poultry production chain through a farm to fork approach.
- A high prevalence of *Salmonella* with predominance of *S*. Infantis, *S*. Kentucky and *S*. Enteritidis was shown. Multi-Drug Resistant *S*. Kentucky and *S*. Infantis strains are circulating all over the chain.
- This study demonstrates the circulation and transmission of identical clones of *Salmonella* throughout the food chain and layer flocks.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Broiler breeder farms, commercial broiler farms, layer farms, slaughterhouses and retail chicken meats (supermarkets and restaurants) were investigated in this study. Sampling was done on faeces and meat and thus did not require any institutional review board approval.

2.1.1 | Farm sample collection

For one year (October 2014/October 2015), a cross-sectional study was performed in 29 broiler breeder farms, 159 broiler production farms and 49 egg laying hen farms. In total, 237 farms randomly chosen from all Lebanese districts were enrolled, with only one flock studied, represented by one environmental sample at each farm during the rearing period. The samples were collected using boot swabs within the poultry house. To perform sampling, pair of sterile elastic cotton socks were worn over the boots and faecal samples were collected by walking through the entire poultry house. Embedded faeces on the cotton socks were put aseptically into sterilized containers and transported within 2 hr to the Lebanese Agricultural Research Institute (LARI) in an ice cooler container for *Salmonella* detection analysis.

2.1.2 | Processing plant sample collection

Over one-year period (June 2015/June 2016), two major poultry processing plants (Slaughter plant A and Slaughter plant B), listed among the top four broiler production plants, covering more than the half of the Lebanese chicken production, were included in this study. Both slaughterhouses were fully automated applying Good Hygiene Practices (GHP) and Good Manufacturing Practices (GMP) systems.

Both companies are either integrated or contracting with rearing private farms providing them with one- day- age chicks ('Ross 308' and 'Hubbard classic' species for processing plant A and B, respectively) and a diet formula. All breeder birds were vaccinated against *S*. Typhimurium and *S*. Enteritidis. The Slaughter plant A is considered the largest slaughterhouse at a national level with a capacity of almost 22,500,000 broilers slaughtered per year. The evisceration was carried out automatically, Peracetic Acid (PAA) was used as antimicrobial in all the processing steps and chilling was achieved by dry air. On the other hand, slaughterhouse B is considered a small-scale poultry processing plant with a capacity of 3,750,000 broilers slaughtered per year. Contrary to slaughterhouse A, the first step of chilling in slaughterhouse B was performed by immersion system with the addition of chlorine (0.3 ppm for 20 min) followed by air chilling for 30 min.

Thirty-eight and six farms were randomly chosen from Slaughter plant A and B, respectively. The number of the farms was representatively taken according to the size of the enterprise. At least one sample was taken from each farm during sampling period (autumn, winter and spring seasons).

During processing, one sample of neck skin of post-chilled carcass and 5 to 10 caeca samples during evisceration (pooled in one sample) were taken randomly from each slaughtered flock. In total, 230 samples were collected, with 202 (101 neck skin and 101 caeca) and 28 (14 neck skin and 14 caeca) from slaughter plant A and B, respectively. Samples were coded A or B with the number of sampling from slaughter plants A and B, respectively. A farm was considered to be *Salmonella*-positive if at least one sample was positive whether in caeca or in neck skins.

2.1.3 | Poultry meat sample collection

For 3 years, from November 2014 until November 2016, 128 samples of whole chicken carcasses and cuts, and 6 samples of liver were chosen randomly from different slaughterhouses covering all Lebanese regions. In parallel, 1907 samples were collected from Lebanese retail shops (supermarkets and restaurants) including 1156 samples of raw chicken parts (133 liver and 1023 whole chicken carcasses and cuts) and 751 samples of marinated chicken meat.

2.1.4 | Avian and human *Salmonella* isolates collection

Avian *Salmonella* isolates from previous outbreaks in Lebanon and imported raw cuts (16 and 30 isolates, respectively) were included in this study.

For comparison purposes, five isolates of clinical *S*. Enteritidis were picked out from the most predominant pulsotype JEGX01.0001 (Fadlallah et al., 2017) collected from a large repository of *Salmonella* strains in the PulseNet laboratory at the American University of Beirut (AUB).

2.2 | *Salmonella* isolation and identification

Salmonella spp. was isolated and identified according to the ISO method NF EN ISO 6579 (2002). Briefly, 25g of sample was homogenized in 225 ml of Buffered Peptone Water (BPW) (Scharlau, Spain). After incubation for 18 hr at 37°C, 1 ml and 0.1 ml of the pre-enrichment suspension were added to 10 ml of Mueller Kauffman Tetrathionate broth (Scharlau, Spain) and 10 ml of Rappaport Vassiliadis Soy broth (Scharlau, Spain) and incubated at 37°C and 41.5°C, respectively. After 24 hr of incubation, 10 µl of each broth was streaked onto Xylose Lysine Desoxycholate (XLD) agar and Salmonella-Shigella agar (SS) plates (Scharlau, Spain) and incubated at 37°C for 24 hr to 48 hr. Typical colonies were further confirmed by API® 20E (Biomerieux, France). Confirmed isolates were further serotyped by slide agglutination using commercial O and H antisera (Remel, UK) in accordance with the Kauffman and White le Minor scheme (2007). One colony was serotyped from each culture-positive sample.

2.3 | Antimicrobial susceptibility testing

From the 672 isolates, 514 were selected for the antimicrobial susceptibility testing (AST) according to the rank prevalence of the first three serotypes, which were *S*. Infantis (n = 204), *S*. Enteritidis (n = 177) and *S*. Kentucky (n = 133). AST was carried out in accordance with the Clinical and Laboratory Standards Institute (CLSI, 2020). The Kirby-Bauer disc diffusion method was firstly performed, for a panel of 26 antimicrobials (Oxoid, Basingstoke, England): ampicillin (AMP-10 µg), amoxicillin-clavulanic acid (AMC-30 µg), piperacillin-tazobactam (TZP-110 µg), cephalothin (KF-30 µg), cefuroxime (CXM-30 µg), cefoxitin (FOX-30 µg), cefotaxime (CTX-30 µg), ceftriaxone (CRO-30 µg), ceftazidime (CAZ-30 µg), ceftiofur (EFT-30 µg), cefepime(FEP-30 µg), imipenem (IPM-10 µg), aztreonam (ATM-30 µg), gentamycin (CN-10 µg), tobramycin (TOB-10 µg), streptomycin (S-10 µg), amikacin(AK-30 µg), netilmicin (NET-30 µg), nalidixic acid (NA-30 µg), ciprofloxacin (CIP-5 µg), norfloxacin (NOR-10 µg), enrofloxacin (ENR-5 µg), trimethoprim (W-5 µg), trimethoprim-sulfamethoxazole (SXT-1.25/23.75 µg), tetracycline (TE-30 µg), chloramphenicol (C-30 µg). MICs for resistant isolates were determined using broth microdilution for the following antimicrobials and breakpoint values: KF (≥32 µg/ml), CXM (≥32 µg/ml), FOX (≥32 µg/ml), CTX (≥4 µg/ml), CRO (≥4 µg/ml), CAZ (≥16 µg/ml), EFT (≥8 µg/ml), CN (≥16 µg/ml), NA (≥32 µg/ml), CIP (≥1 µg/ml), NOR (≥16 µg/ml), ENR (≥2 µg/ml). *Escherichia coli* ATCC® 25,922™ was used as a quality control strain. Antimicrobial resistance to more or equal to 3 classes was considered multi-drug resistance (MDR).

2.4 | Pulse-field gel electrophoresis

A pulsed-field gel electrophoresis (PFGE) analysis of isolates of *S*. Kentucky (n = 97), *S*. Infantis (n = 64), and *S*. Enteritidis (n = 53)

was performed using an XbaI restriction enzyme according to the protocol described by (Kérouanton et al., 2007). The isolates were randomly chosen according to their representativeness of all stages of the poultry production chain (from farm to fork) and the different antibiotic resistance profiles. This represents 42% of all isolates tested for antibioresistance.

Similarities of PFGE profiles were determined with Bionumerics 7.6 software (Applied Maths, Kortrijk, Belgium) using the Dice Coefficient and dendrograms were generated graphically by using unweighted pair group method with arithmetic mean (UPGMA) with a 1% tolerance limit and 1% optimization. *Salmonella* Braenderup H9812 was used as a molecular marker'.

2.5 | Statistical analysis

The differences of *Salmonella* spp. prevalence between the slaughterhouses A and B, and among samples were evaluated by the Chi-Square test using the software R (R × 64 version 3.4.3 (2017-11-30). *p*-values ≤.05 were considered statistically significant.

3 | RESULTS

3.1 | Prevalence of *Salmonella* throughout the broiler food chain and laying hen flocks

A total of 30% of the analysed farms was positive for *Salmonella* with a prevalence of 31%, 31.4% and 24.5% in the breeder farms, broiler farms and laying hen farms, respectively (Table 1). In addition, the slaughterhouses showed a prevalence of 35.8% in raw chicken parts compared to 22.4% in retail market.

The overall prevalence of *Salmonella* spp. estimated from caeca sampling in the two slaughterhouses was 81.8% (36/44) indicating the prevalence at farm level (*Salmonella* spp. was isolated at least once during seasonal sampling) and 34.8% at sample level (80/230) having 47.8% of positive caeca (55/115) and 21.7% of positive neck skin (25/115) (Table 2). Caeca prevalence was quite similar in both Slaughterhouses A and B ($p > .05$) on the contrary to neck skin where contamination in slaughterhouse B ($p < .05$) was higher than slaughterhouse A. Moreover, the seasonal effect was not significant ($p > .05$) for both slaughterhouses, the prevalence in winter was 41.7%, in fall 35.7% and in summer 28.4% (Table 2).

3.2 | Distribution of *Salmonella* serotypes

From a total of 672 confirmed *Salmonella* isolates, 23 serotypes were identified as *S*. Infantis (32.9%), *S*. Enteritidis (28.4%) and *S*. Kentucky (21.4%) being the most predominant ones (Table 3). Both *S*. Kentucky and *S*. Infantis were disseminated all along the broiler production chain; however, *S*. Kentucky extended its prevalence to the laying hen farms. *S*. Enteritidis was isolated from all stages of the poultry production chain except from the 2 investigated slaughterhouses A and B.

S. Enteritidis (66.7%, 44.4% and 38%), *S*. Kentucky (16.7%, 33.3% and 22%) and *S*. Blockely (16.7%, 22.2% and 6%) were found in the laying hens, in the breeder and in the broiler farms, respectively. In addition to these three serotypes, *S*. Infantis (22%), *S*. Emek (8%) and *S*. Seftenberg (4%) were isolated from the broiler farms.

The *Salmonella* serotypes differed between the two slaughter plants A and B surveyed. In slaughterhouse A, from the 65 *Salmonella* isolates (48 from caeca and 17 from neck skin), four serotypes were identified. *S*. Kentucky (52.3%) and *S*. Infantis (29.2%) were predominant and repeatedly isolated from caeca and neck skin through the seasons sampling. Within slaughterhouse B, four serotypes were identified from the 15 *Salmonella* isolates (8 from neck skin and 7 from caeca). *S*. Hadar (46.7%) was the predominant one, followed by *S*. Infantis (26.7%), *S*. Istanbul (13.3%) and *S*. Aarhus (13.3%), with the latter was only isolated from neck skin.

In chicken meat collected from slaughterhouses, five *Salmonella* serotypes were identified belonged mainly to *S*. Enteritidis (66.7%), *S*. Infantis (18.8%) and *S*. Kentucky (10.4%). The highest diversity was observed at retail level where 21 *Salmonella* serotypes were recovered with *S*. Infantis (41.2%), *S*. Enteritidis (26.2%) and *S*. Kentucky (28.8%) the most predominant ones.

In addition, five serotypes were identified from the 30 *Salmonella* isolates of imported chicken cuts with *S*. Heidelberg (83.3%) the most predominant.

Among the avian *Salmonella* isolates isolated from previous outbreaks, *S*. Enteritidis (93.8%) was the most frequent serotype (Table 3).

TABLE 1 Sample type and prevalence of *Salmonella* spp at different points of poultry production chain

Source type	Sample type	Number of samples	Number of contaminated samples/(%)
Egg laying hens farm	Faeces	49	12 (24.5%)
Broiler breeders farm	Faeces	29	9 (31%)
Broiler farm	Faeces	159	50 (31.4%)
Total farms		237	71 (30%)
Slaughter house	Raw chicken parts	134	48 (35.8%)
Retail (restaurant and supermarket)	Raw chicken parts	1907	427 (22.4%)

TABLE 2 Number of contaminated/collected samples and the percentage of occurrence of *Salmonella* spp in slaughter plants A and B at different seasons

Samples type	Summer	Autumn	Winter	Total samples	Total farms
Slaughter plant A					
Caeca	13/38 (34.2%)	16/30 (53.3%)	19/33 (57.6%)	48/101 (47.5%)[c]	31/38 (81.6%)[b]
Neck skin	6/38 (15.8%)	5/30 (16.7%)	6/33 (18.2%)	17/101 (16.8%)[a]	
Total samples	19/76 (25%)	21/60 (35%)	25/66 (37.9%)	65/202 (32.2%)	
Slaughter plant B					
Caeca	3/6 (50%)	1/5 (20%)	3/3 (100%)	7/14 (50%)[c]	5/6 (83.3%)[b]
Neck skin	3/6 (50%)	3/5 (60%)	2/3 (66.7%)	8/14 (57.1%)[a]	
Total samples	6/12 (50%)	4/10 (40%)	5/6 (83.3%)	15/28 (53.6%)	
Total Slaughter Plants					
Caeca	16/44 (36.4%)	17/35 (48.6%)	22/36 (61.1%)	55/115 (47.8%)	36/44 (81.8%)
Neck skin	9/44 (20.5%)	8/35 (22.9%)	8/36 (22.2%)	25/115 (21.7%)	
Total samples	25/88 (28.4%)[d]	25/70 (35.7%)[d]	30/72 (41.7%)[d]	80/230 (34.8%)	

[a]Indicates a significant difference of *Salmonella* prevalence in neck skin between slaughterhouses A and B.
[b]Indicates no significant differences of *Salmonella* prevalence between the two slaughterhouses at farm level.
[c]Indicates no significant differences of *Salmonella* prevalence in caeca between the two slaughterhouses.
[d]Indicates no significant differences of *Salmonella* prevalence between the seasons in the two slaughterhouses.

3.3 | Antimicrobial resistance phenotypes

Five hundred and fourteen *Salmonella* isolates belonging to the 3 most predominant serotypes throughout the whole broiler food chain and laying hen flocks, *S*. Infantis (n = 204), *S*. Enteritidis (n = 177) and *S*. Kentucky (n = 133) were subjected to antimicrobial susceptibility testing. A multitude of antimicrobial resistance patterns were detected, where from 1 up to 6 antimicrobial classes were involved. High resistance against nalidixic acid was commonly observed: *S*. Enteritidis (98.9%), *S*. Intantis (99.5%) and *S*. Kentucky (100%). *S*. Enteritidis was the most sensitive with a very low level of MDR (5.1%). On the other hand, *S*. Infantis isolates showed a remarkable high resistance to tetracycline (99%) and streptomycin (88.2%), and to a lesser extent, to trimethoprim (2.4%) and trimethoprim-sulfamethoxazole (1%) (Figure 1). The majority of this serotype (89.7%) were MDR with 14 different antimicrobial profiles having the 'S-NA-TE' pattern (71.6%) as the most predominant one circulating in all over the broiler food chain (Tables 4 and 5).

In parallel, all the *S*. Kentucky isolates were resistant to quinolones and fluoroquinolones with very high ciprofloxacin MIC levels observed (6.25–>32 μg/ml). Very high resistance was found to ampicillin (71.4%), amoxicillin–clavulanic acid (56.4%), tetracycline (58.7%), streptomycin (54.9%) and gentamycin (53.4%) (Figure 1). Resistance against cefoxitin and third-generation cephalosporins (cefotaxime, ceftriaxone, ceftazidime and ceftiofur) was observed in 9 isolates representing 6.8% of the total *S*. Kentucky and were isolated from slaughterhouse A (n = 2) and retail (n = 7). For only one isolate (K38), although initial disc diffusion test revealed resistance to cefoxitin, broth microdilution demonstrated a reduced susceptibility to FOX (MIC = 12.5 μg/ml) comparing to the other FOX resistant isolates (MIC ≥ 200 μg/ml).

A very large diversity of antimicrobial resistance profile (n = 36) was observed among this serotype with 27 (62.4% of the isolates) considered as a MDR patterns disseminated along the food chain (Tables 4 and 5).

3.4 | Pulse-field gel electrophorese

Among the 97 isolates of *S*. Kentucky, 10 different pulsotypes stand out, with a diversity index of 0.767. Genotyping with one restriction enzyme showed 5 different clusters with a degree of similarities ≥95.7% between all *S*. Kentucky isolates. The main cluster includes 35 *S*. Kentucky isolates and covered the broiler food chain, from broiler breeder farm (n = 1), broiler farm (n = 1), slaughterhouse A (skin neck = 3, caeca = 13) to retail (n = 17) (Figure 2).

With a diversity index of 0.966, *S*. Infantis showed a great diversity among the isolates and established a contamination at all steps of the broiler production and not only at the slaughterhouse. Among the 64 isolates, 36 pulsotypes were distinguished. Interestingly, associate isolates with 100% of similarity originated from farm, slaughterhouse, supermarket and restaurant (Figure 3).

Seven pulsotypes were demonstrated for *S*. Enteritidis with a 0.369 diversity index. A relationship between several isolates from farm to fork is also present for this serotype. One dominant profile (grouping 80% of the isolates) contains sporadic human and poultry isolates from different sources: broiler breeder farm, layer farm, broiler farm, slaughterhouses, retail and food suspected of intoxication (Figure 4).

TABLE 3 *Salmonella* serotypes diversity isolated along the chicken production chain

Serotype	Total number	Egg hens Layer farms	Broiler breeder farms	Broiler farms	Slaughter plant A	Slaughter plant B	Slaughterhouse (meat)	Retail	Suspected food (Intoxication)	Imported
S. Infantis	221 (32.9%)			11 (22%)	19 (29.2%)	4 (26.7%)	9 (18.8%)	176 (41.2%)	1 (6.3%)	1 (3.3%)
S. Enteritidis	191 (28.4%)	8 (66.7%)	4 (44.4%)	19 (38%)			32 (66.7%)	112 (26.2%)	15 (93.8%)	1 (3.3%)
S. kentucky	144 (21.4%)	2 (16.7%)	3 (33.3%)	11 (22%)	34 (52.3%)		5 (10.4%)	89 (20.8%)		
S. Heidelberg	31 (4.6%)							6 (1.4%)		25 (83.3%)
S. Newport	15 (2.2%)				2 (3.1%)		1 (2.1%)	12 (2.8%)		
S. Blockley	11 (1.6%)	2 (16.7%)	2 (22.2%)	3 (6%)	1 (1.5%)			3 (0.7%)		
S. Typhimurium	9 (1.3%)							9 (2.1%)		
S. Hadar	9 (1.3%)					7 (46.7%)	1 (2.1%)	1 (0.2%)		
S. Emek	7 (1%)			4 (8%)				3 (0.7%)		
S St paul	4 (0.6%)							2 (0.5%)		2 (6.7%)
S. Munster	3 (0.4%)							3 (0.7%)		
S. Aarhus	3 (0.4%)					2 (13.3%)		1 (0.2%)		
S. Branderup	2 (0.3%)							2 (0.5%)		
S. Virginia	2 (0.3%)							1 (0.2%)		1 (3.3%)
S. Istanboul	2 (0.3%)					2 (13.3%)				
S. Senftenberg	2 (0.3%)			2 (4%)						
S. Glostrup	1 (0.1%)							1 (0.2%)		
S. Mbandaka	1 (0.1%)							1 (0.2%)		
S. Anatum	1 (0.1%)							1 (0.2%)		
S. Montevideo	1 (0.1%)							1 (0.2%)		
S. Agona	1 (0.1%)							1 (0.2%)		
S. Rissen	1 (0.1%)							1 (0.2%)		
S. Lomita	1 (0.1%)							1 (0.2%)		
NT	9 (1.3%)				9 (13.8%)					
Total	672	12	9	50	65	15	48	427	16	30

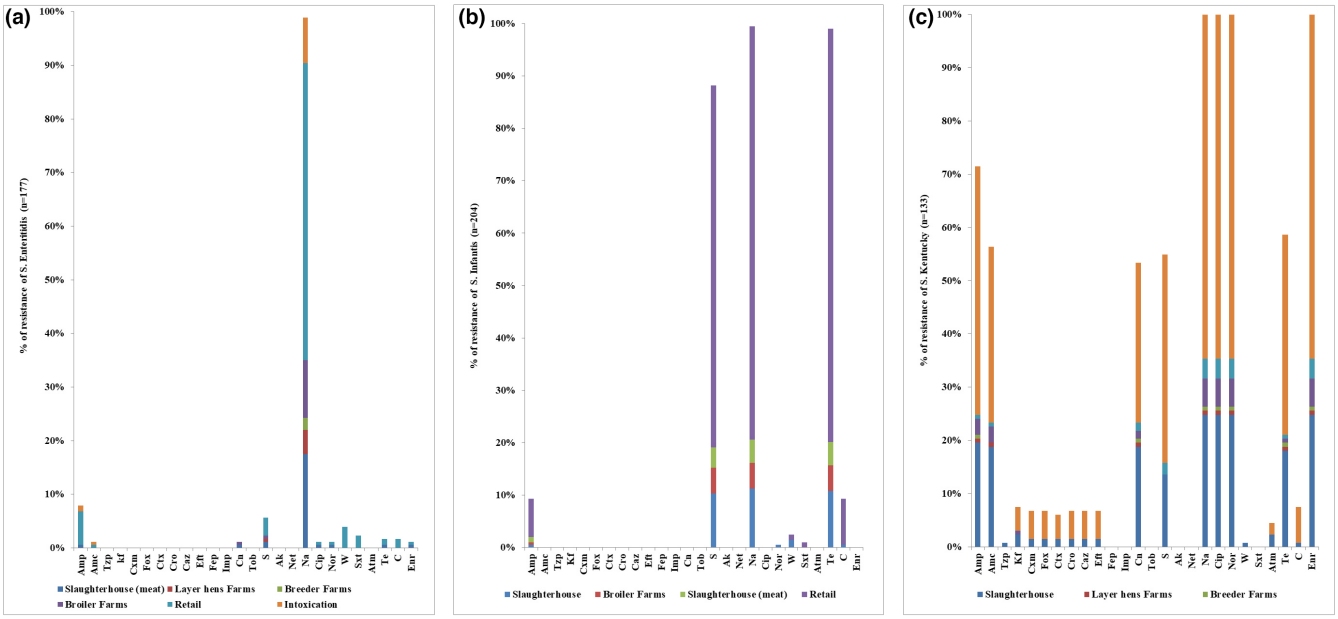

FIGURE 1 Percentage of antimicrobial resistance of *S*. Enteritidis (a), *S*. Infantis (b) and *S*. Kentucky (c) from farms, slaughterhouses and retail. The code of antibiotics are: Ampicillin (AMP), amoxicillin-clavulanic acid (AMC), piperacillin-tazobactam (TZP), cefalothin (KF), cefuroxime (CXM), cefoxitin (FOX), cefotaxime (CTX), ceftriaxone (CRO), ceftazidime (CAZ), ceftiofur (EFT), cefepime (FEP), imipenem (IMP), gentamycin (CN), tobramycin (TOB), streptomycin (S), amikacin (AK), netilmycin (NET), nalidixic acid (NA), ciprofloxacin (CIP), norfloxacin (NOR), trimethoprim (W), trimethoprim-sulfamethoxazole (SXT), aztreonam (ATM), tetracycline (TE), chloramphenicol (C), enrofloxacin (ENR)

4 | DISCUSSION

The overall prevalence of *Salmonella* in the farms is 30%. Infected breeder farms (31%) and layers (24.5%) are of great concern since contamination can be disseminated to commercial broiler flocks and eggs, respectively, via vertical or horizontal transmission. *Salmonella* prevalence in broilers (31.4%) is similar to previous studies done in developing countries such as Algeria (Djeffal et al., 2017). However, it is exceedingly higher than reports from the EU where, in the context of mandatory national Control Programs, the reported flock prevalence were of 1.47%, 2.6% and 3.71% in breeding flocks, broilers and laying hens, respectively (European Food Safety Authority/European Centre for Disease Prevention & Control, 2017). This high occurrence could be attributed to the absence of *Salmonella* reduction plan at the farm level in Lebanon.

The high overall prevalence of 47.8% of *Salmonella* isolated from caeca suggesting that the major source of contamination is mainly at the farm level (fully integrated) rather than at slaughterhouse is in agreement with other studies (Zhu et al., 2017). The lower prevalence of *Salmonella* observed on neck skins from slaughterhouse A (16.83%) comparing to slaughterhouse B (57%) highlighted the differences in their practices: indeed, the first one, in line with the high number of slaughtered poultry, performed air chilling with Peracetic Acid (PAA) (Slaughterhouse A) while the second performed chilling by immersion with chlorine (0.3 ppm) (Slaughterhouse B). In fact, carcass chilling is considered a critical step to avoid the cross-contamination of pathogens. Commercial immersion chilling showed a potential cross-contamination risk (Sukted et al., 2017). On the other hand using chlorine with the presence of high organic materials during the processing often reduces its antimicrobial efficacy (Paul et al., 2017). Another antimicrobials such as PAA, used in Slaughterhouse A, were often chosen and have proved to be more effective in chillers (Blevins et al., 2017).

The retail contamination is lower than other regions in Asia as reported in Japan (54.1%) (Shigemura et al., 2018) and in Korea (42%) (CHOI et al., 2015). This rate is quite steady in Lebanon when compared to a previous study carried out by our institute with 41.6% of contamination in chicken carcass in slaughterhouses (El Hage, 2013 *unpublished data*).

Serotype diversity, higher at the retail level, 21 serotypes compared to only three at the farm level, indicated a higher risk of other contaminating sources that should be investigated further. Our three main serotypes were in accordance with the poultry-associated *Salmonella* serotypes distribution worldwide. *S*. Enteritidis and *S*. Infantis were still among the most prevalent serotypes in laying hens and broilers and broiler meat, respectively, in Europe (European Food Safety Authority/European Centre for Disease Prevention & Control, 2016). *S*. Kentucky ranks among the 12 most prevalent poultry-associated *Salmonella* serotypes worldwide (Shah et al., 2017).

The type of broiler species, the management system adopted, rearing at farm level and its geographical location might explain the obviously different *Salmonella* serotypes diversity in the two slaughterhouses, where only *S*. Infantis is detected as a common serotype. It seems that horizontal contamination of this serotype has occurred from the broiler farm level until retail level. Although being classified as the fourth serotype causing human salmonellosis in the EU and predominating in local poultry production, it could not be associated

TABLE 4 Antimicrobial resistance patterns of *S*. Enteritidis, *S*. Infantis and *S*. Kentucky isolates

Serotypes	Antimicrobial resistance patterns	Laying hen farms	Breeder farms	Broiler farms	Slaughterhouse A and B	Slaughterhouse (meat)	Retail	Total	%
Salmonella Enteritidis	Pan susceptible					1	1	2	1.1
	NA	8	4	19		28	99	156	88.1
	NA-W						1	1	0.6
	S-NA	1		1				2	1.1
	AMP-NA					1	4	5	2.8
	S-NA-TE					1		1	0.6
	AMP-AMC-NA						1	1	0.6
	AMP-S-NA-C						1	1	0.6
	AMP-S-NA-W						2	2	1.1
	AMP-AMC-S-NA-W						1	1	0.6
	AMP-S-NA-W-SXT						1	1	0.6
	CN-S-NA-CIP-NOR-ENR					1		1	0.6
	AMP-S-NA-W-SXT-C						1	1	0.6
	AMP-NA-W-SXT-TE-C						1	1	0.6
	AMP-NA-CIP-NOR-TE-ENR						1	1	0.6
Salmonella Infantis	NA						2	2	1.0
	TE						1	1	0.5
	NA-TE				1	1	16	18	8.8
	S-NA-C				1			1	0.5
	NA-TE-C						2	2	1.0
	S-NA-TE			9	16	6	115	146	71.6
	S-NA-TE-C						10	10	4.9
	S-NA-W-TE				2			2	1.0
	AMP-NA-TE-C						1	1	0.5
	AMP-S-NA-TE			1	2	2	9	14	6.9
	NA-NOR-W-TE				1			1	0.5
	AMP-S-NA-TE-C						4	4	2.0
	S-NA-W-SXT-TE						1	1	0.5
	AMP-S-NA-W-SXT-TE-C						1	1	0.5
Salmonella Kentucky	NA-CIP-NOR-ENR			2	2	2	6	12	9.0
	S-NA-CIP-NOR-ENR				1	1	11	13	9.8
	CN-NA-CIP-NOR-ENR			1	2		1	4	3.0
	NA-CIP-NOR-TE-ENR				1		1	2	1.5
	AMP-NA-CIP-NOR-ENR						2	2	1.5
	S-NA-CIP-NOR-C-ENR						1	1	0.8
	CN-S-NA-CIP-NOR-ENR					1	2	3	2.3
	AMP-AMC-S-NA-CIP-NOR-ENR						1	1	0.8
	CN-NA-CIP-NOR-TE-ENR						1	1	0.8
	CN-S-NA-CIP-NOR-TE-ENR				1			1	0.8
	AMP-S-NA-CIP-NOR-ENR						1	1	0.8
	AMP-NA-CIP-NOR-TE-ENR						1	1	0.8
	AMP-AMC-NA-CIP-NOR-ENR			2	3		6	11	8.3
	AMP-S-NA-CIP-NOR-TE-ENR						7	7	5.3
	AMP-CN-NA-CIP-NOR-TE-ENR		1				2	3	2.3

TABLE 4 (Continued)

Serotypes	Antimicrobial resistance patterns	Laying hen farms	Breeder farms	Broiler farms	Slaughterhouse A and B	Slaughterhouse (meat)	Retail	Total	%
	AMP-AMC-CN-NA-CIP-NOR-ENR						1	1	0.8
	AMP-AMC-KF-NA-CIP-NOR-ENR			1				1	0.8
	AMP-AMC-NA-CIP-NOR-TE-ENR						3	3	2.3
	AMP-CN-S-NA-CIP-NOR-TE-ENR				1		6	7	5.3
	AMP-AMC-CN-S-NA-CIP-NOR-ENR				1		1	2	1.5
	AMP-AMC-S-NA-CIP-NOR-TE-ENR						3	3	2.3
	AMP-AMC-CN-NA-CIP-NOR-TE-ENR	1		1	3		6	11	8.3
	AMP-AMC-KF-NA-CIP-NOR-TE-ENR				1			1	0.8
	AMP-AMC-CN-NA-CIP-NOR-TE-C-ENR						1	1	0.8
	AMP-AMC-CN-S-NA-CIP-NOR-TE-ENR				11	1	7	19	14.3
	AMP-AMC-CN-NA-CIP-NOR-ATM-TE-ENR				1			1	0.8
	AMP-AMC-CN-S-NA-CIP-NOR-TE-C-ENR				1		6	7	5.3
	AMP-AMC-CN-S-NA-CIP-NOR-W-TE-ENR				1			1	0.8
	AMP-AMC-TZP-CN-NA-CIP-NOR-TE-ENR				1			1	0.8
	AMP-AMC-CN-S-NA-CIP-NOR-TE-ENR						2	2	1.5
	AMP-AMC-KF-CXM-FOX-CTX-CRO-CAZ-EFT-NA-CIP-NOR-ENR						2	2	1.5
	AMP-AMC-KF-CXM-FOX-CTX-CRO-CAZ-EFT-NA-CIP-NOR-C-ENR						1	1	0.8
	AMP-AMC-CXM-FOX-CRO-CAZ-EFT-CN-S-NA-CIP-NOR-ATM-TE-ENR						1	1	0.8
	AMP-AMC-KF-CXM-FOX[a]-CTX-CRO-CAZ-EFT-CN-S-NA-CIP-NOR-TE-ENR						1	1	0.8
	AMP-AMC-KF-CXM-FOX-CTX-CRO-CAZ-EFT-CN-NA-CIP-NOR-ATM-TE-ENR				1			1	0.8
	AMP-AMC-KF-CXM-FOX-CTX-CRO-CAZ-EFT-CN-S-NA-CIP-NOR-ATM-TE-ENR				1		2	3	2.3

Note: The code of antibiotics are: ampicillin (AMP), amoxicillin-clavulanic acid (AMC), piperacillin-tazobactam (TZP), cefalothin (KF), cefuroxime (CXM), cefoxitin (FOX), cefotaxime (CTX), ceftriaxone (CRO), ceftazidime (CAZ), ceftiofur (EFT), gentamycin (CN), streptomycin (S), nalidixic acid (NA), ciprofloxacin (CIP), norfloxacin (NOR), trimethoprim (W), aztreonam (ATM), tetracycline (TE), chloramphenicol (C), enrofloxacin (ENR).

[a]Categorized as Resistant according to disc diffusion method and reduced susceptibility according to broth microdilution.

TABLE 5 Antimicrobial Resistance, multi-drug resistance and extended-spectrum cephalosporin occurrence of the main serotypes isolated in this study

Serovars	Number of antimicrobial classes/(%)							Total MDR	ESCs
	0	1	2	Multi drug Resistance					
				3	4	5	6		
Salmonella Enteritidis (*n* = 177)	2 (1.1%)	156 (88.1%)	10 (5.6%)	2 (1.1%)	5 (2.8%)	2 (1.1%)	0 (0%)	9 (5.1%)	0 (0%)
Salmonella Infantis (*n* = 204)	0 (0%)	3 (1.5%)	18 (8.8%)	150 (73.5%)	28 (13.7%)	4 (2%)	1 (0.5%)	183 (89.7%)	0 (0%)
Salmonella Kentucky (*n* = 133)	0 (0%)	12 (9%)	38 (28.6%)	14 (10.5%)	60 (45.1%)	9 (6.8%)	0 (0%)	87 (62.4%)	9 (6.8%)
Total (*n* = 514)	2 (0.4%)	171 (33.3%)	66 (12.8%)	166 (32.3%)	93 (18.1%)	15 (2.9%)	1 (0.2%)	279 (53.5%)	9 (1.8%)

to foodborne outbreak in Lebanon (MOPH/Pulse Net report, 2015 *unpublished data*).

The high prevalence of *S*. Enteritidis, especially at breeders and layers farms is very concerning. This serotype, leading cause of human salmonellosis in Lebanon (MOPH/Pulse Net report, 2015 *unpublished data*) and in the world (EFSA/ECDC, 2016; Foley et al., 2011), is known to be vertically transmitted and, therefore, leads to the contamination of broiler flocks and eggs (Cox et al., 2000). In the EU, outbreaks due to *S*. Enteritidis in eggs have caused the highest number of outbreak cases in 2016. Moreover, when performing PFGE analysis on *S*. Enteritidis isolates, in addition to farms, retail and human isolates shared the same pulsotype at a rate of 80%. This same pulsotype has been circulating in Lebanon since 2010 and was linked to three outbreaks (Saleh et al., 2011) and isolated in clinical and food samples in 2017 (Fadlallah et al., 2017). Previous studies have demonstrated that this serotype is highly clonal (Campioni et al., 2012; Fardsanei et al., 2017). It is noteworthy to mention that PFGE presents challenges in the differentiation of genetically homogeneous serotypes such as *S*. Enteritidis. Approximately 45% of this serotype reported to Pulse Net exhibit the same PFGE *Xba*I pattern, although many of these isolates are not epidemiologically related (Tang et al., 2019). It became sure that a more precise, reliable but more expensive method such as Whole Genome Sequencing (WGS) will be the reference tool to track outbreaks and identify *Salmonella* genomic diversity. But considering the objective of this study that captures the general status, PFGE, which is still a 'gold standard' and a valuable tool for foodborne pathogen characterization, has been used to genetically identify *Salmonella* isolates. The presence of *S*. Enteritidis was confirmed along the broiler production chain and layer flocks; however, it was not detected in both slaughterhouses A and B. This might be due to the vaccination programs against *S*. Enteritidis and *S*. Typhimurium at the broiler breeder level, which is known to be effective in controlling *Salmonella*.

One of our major findings is the high prevalence of *S*. Kentucky circulating all over the poultry production chain, and it has been the most predominant in the biggest slaughterhouse in the country (52.3%). In fact, this serotype is highly present in poultry worldwide (Shah et al., 2017) but not commonly associated with human illness in Lebanon (MOPH/Pulse Net report, 2015 unpublished data) and in USA (CDC, 2017) and only 1% of human salmonellosis in EU (European Food Safety Authority/European Centre for Disease Prevention & Control, 2016). Its capacity to grow in moderate acidic environment provides it an advantage over other serotypes to proliferate in chicken caecum (Foley et al., 2013). The route to broiler flocks' contamination remains unclear. Mostly horizontally transmitted, (Papadopoulou et al., 2009) indicated that protein concentrates in animal feed are source of contamination by *S*. Kentucky.

Most isolates from imported chicken were *S*. Heidelberg, suggesting that the source of contamination of this serotype was derived from Brazilian chicken meat products. This was the case in the EU where contaminated chicken cuts, notified by the Rapid Alert System for Food and Feed were imported from Brazil (RASFF, 2017).

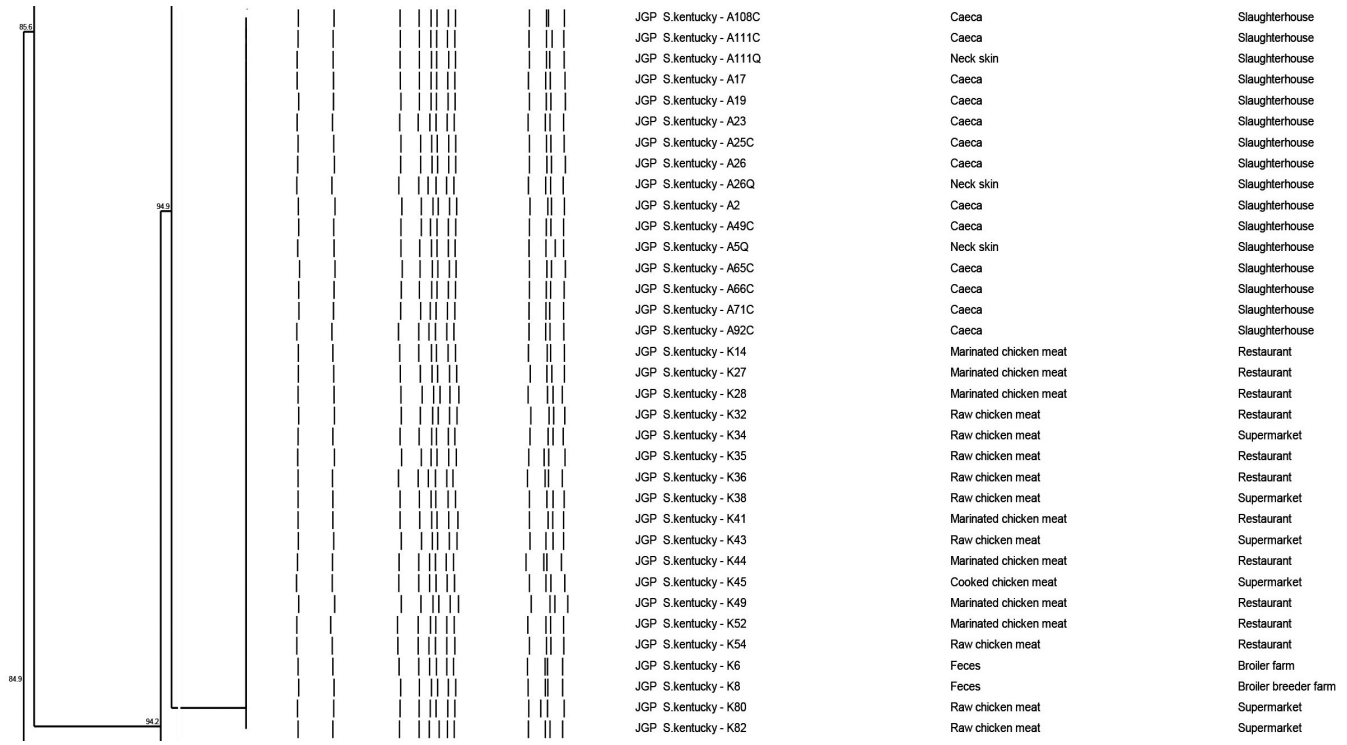

FIGURE 2 Macrorestriction patterns of *S*. Kentucky using the Dice coefficient, and the dendrograms were generated graphically by using unweighted pair group method with arithmetic mean (UPGMA). The codes A, B and K designate the *Salmonella* isolates from slaughterhouse A, slaughterhouse B and retail respectively. The letters C or Q are related to caeca or neck skin respectively

In Lebanon, fluoroquinolones (enrofloxacin), third-generation cephalosporins (ceftiofur) and trimethoprim are widely used in the therapy of poultry production. In this investigation, an extremely high rate of nalidixic acid resistance and highly worrisome MDR among the isolates were obtained. Since the introduction of the fluoroquinolone class in poultry production, nalidixic acid resistance of *Salmonella* has been often reported worldwide (Ferri et al., 2017; Gouvêa et al., 2015). This is of great concern since this resistance may be an indicator of reduced susceptibility to other quinolones of clinically great importance such as ciprofloxacin (Choi et al., 2005). *S*. Enteritidis showed the lowest resistance, which is in accordance with international findings (Michael & Schwarz, 2016). Furthermore, our results showed that antimicrobial resistance persists in all stages of the broiler production chain and layer flocks and has increased in all *Salmonella* serotypes tested at the end of the production chain, especially at retail level, suggesting that these serotypes may gained resistance from resistance genes in the environment (Ferri et al., 2017) and, therefore, the dissemination of more AMR to consumers.

Despite the recent emergence of antimicrobial resistance among *S*. Infantis in Europe, it is still reported as pan-susceptible in USA poultry (Shah et al., 2017). High incidence of MDR *S*. Infantis (89.7%) having the 'S-NA-TE' pattern (71.6%) might be related to a similar clonal spread in broiler and humans detected in Hungary (Nógrády et al., 2007). The typically emerging pattern 'NA-S-SUL-TE' became widely disseminated in European countries (Nógrády et al., 2012) due to the presence of pESI (plasmid emerging *S*. Infantis) (Aviv et al., 2014) conferring high antimicrobial resistance, virulence and stress tolerance. High genetic relatedness (87.8%) has been found among these isolates in accordance to other studies (Franco et al, 2015; Vinueza-Burgos et al., 2016) suggesting that this strain is clonal. PFGE analysis with high rate of individual subtypes, especially at the retail level, suggests frequent possibilities of cross-contamination. In addition, similar PFGE patterns have been detected across isolates from different sources where slaughterhouse A was mainly implicated, suggesting that this slaughterhouse may be the source of *Salmonella* Infantis contamination.

All *S*. Kentucky isolates were highly ciprofloxacin-resistant (MIC level: 6.25–>32 μg/ml) and showed large, diverse antimicrobial resistance profiles. Our *S*. Kentucky isolates in both MDR, mainly 'AMP-AMC-CN-S-NA-CIP-NOR-TE-ENR' and AMR (AMP, AMC, TE, S, CN) are very similar to the emerging ST198-X1 strain and coherent with the rapid and extensive global epidemic Ciprofloxacin resistant ability of this subtype described by Le Hello (2013). The source of this contamination is very variable, (Le Hello et al., 2011) strengthened on poultry as the main niche in Africa (Ethiopia, Nigeria Morocco,) and Europe (Poland, Germany and France) other on reptiles (Zajac et al., 2013) and the environment (Le Hello et al., 2013). It is also shown that nine different isolates of *S*. Kentucky are ESCs comparable to those detected in the Mediterranean basin (Le Hello et al., 2013). Two of them (A 66C-B108C), were isolated from slaughterhouse A and seven (K12, K24, K31, K32, K38, K43, K48) from retail, indicating that

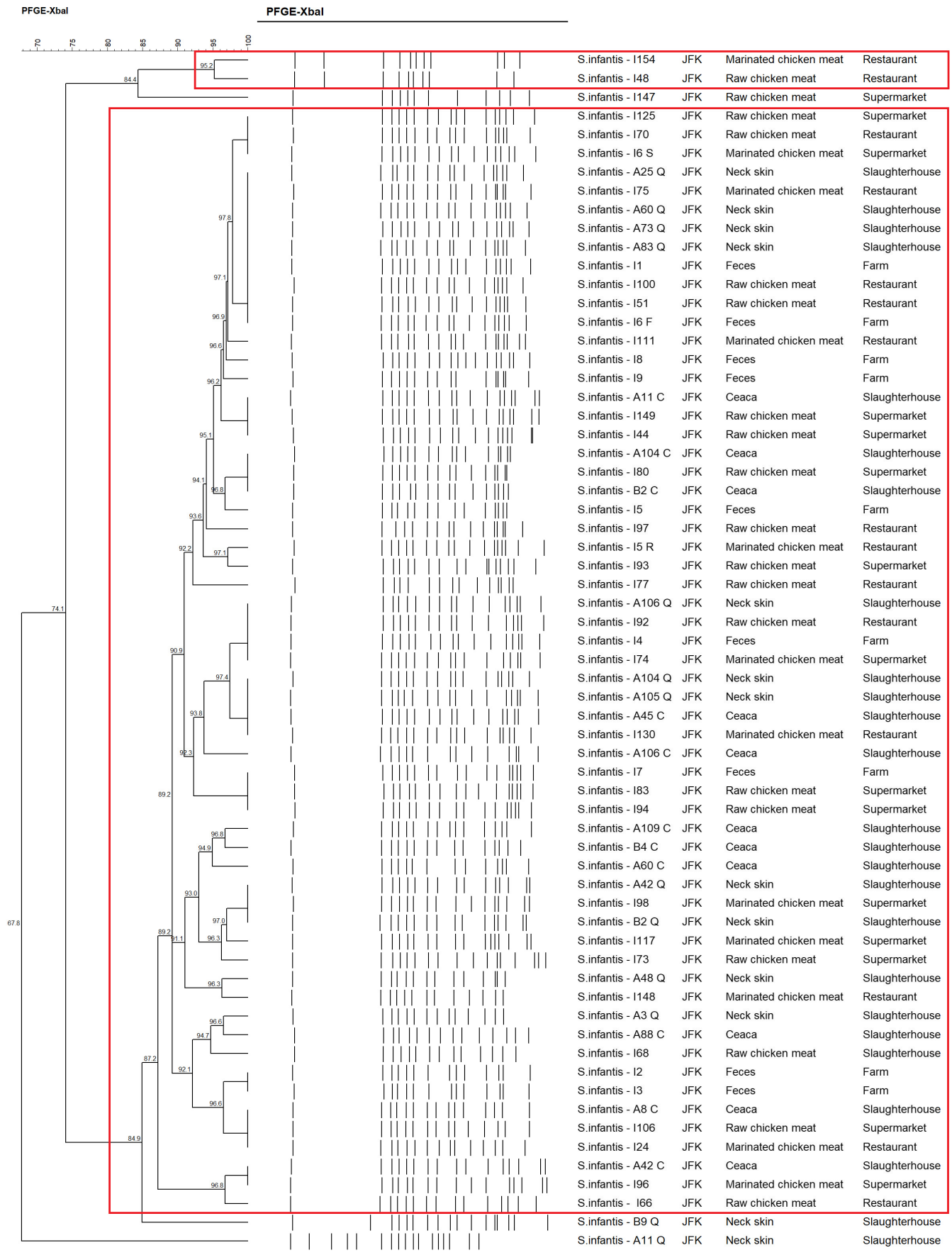

FIGURE 3 Macrorestriction patterns of *S*. Infantis using the Dice coefficient and the dendrograms were generated graphically by using unweighted pair group method with arithmetic mean (UPGMA). The code A, B and I designate the *Salmonella* isolates from slaughterhouse A, slaughterhouse B and retail. The letters C or Q are related to caeca or neck skin respectively

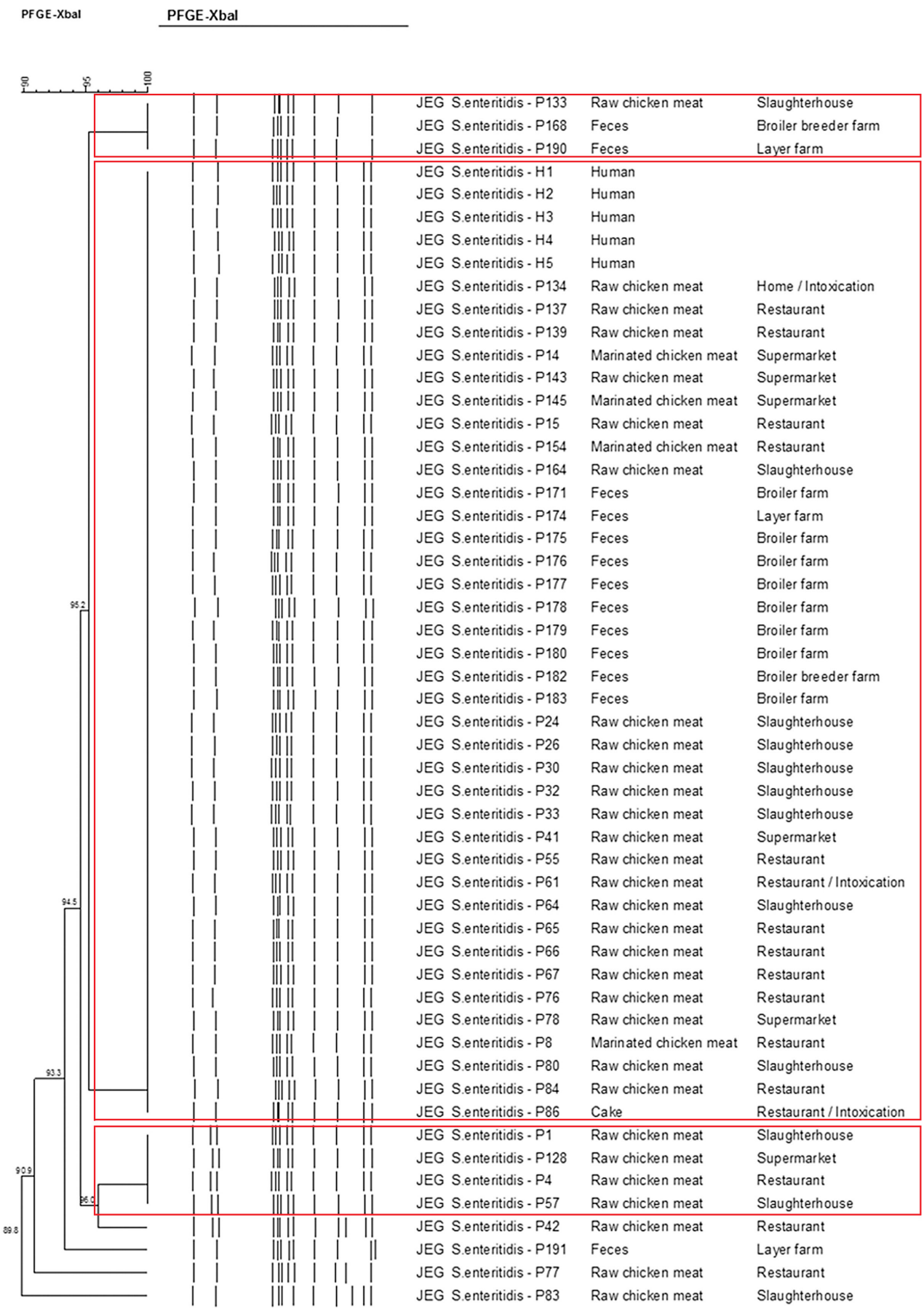

FIGURE 4 Macrorestriction patterns of *S*. Enteritidis using the Dice coefficient and the dendrograms were generated graphically by using unweighted pair group method with arithmetic mean (UPGMA). The code P designate the *Salmonella* isolates

this slaughterhouse is the causative dissemination of ESCs *S*. Kentucky. In addition, according to PFGE analysis, these isolates are highly related, proposing that this strain is also clonal. Isolates from farms (layers or /and broiler breeders or/ and broilers), slaughterhouse A and retail were grouped within one pulsotype suggesting that S. Kentucky is circulating throughout the broiler food chain and layer flocks. It should be noted that slaughterhouse A is a fully integrated poultry establishment and, therefore, its role in the dissemination of *S*. Kentucky is important.

It is foreseen that the status quo of the serotypes diversity and their antibiotic resistance in the poultry production chain in Lebanon would not be changed or evolved since no countermeasures were taken during the period preceding the sample collection.

5 | CONCLUSION

This is the first integrated approach in Lebanon trying to answer decision makers concerns on *Salmonella* control. Not only a high rate of *Salmonella* incidence was detected, but also a multitude of MDR isolates and clones against critical antibiotics were observed along the food chain. The miss and/over uses of uncontrolled drugs in Lebanese animal production are the leading origin of emergent MDR bacteria, but also the circulation of resistant isolates from other countries via traveling and good trades. Further detailed investigation is needed to accurately characterize and subtype *Salmonella* with pinpoint methods like WGS. To control this public health risk, it is of utmost importance to review the current national food safety strategy and to implement effective measures aiming to reduce the prevalence throughout the chain and the transmission of this pathogen to humans.

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CONFLICT OF INTEREST

No conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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