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ORIGINAL ARTICLE

Characterization of *Salmonella* isolated from organically reared poultry located in the same longitude with three distinct seasonal characteristics

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Abstract

This study is designed to determine *Salmonella* prevalence in organic poultry farms and slaughterhouse in three different regions with distinct seasonal characteristics. *Salmonella* strains were isolated from organic reared poultry farm environment (water, feed, and feces) and poultry meat samples (neck skin and breast meat). Antibiotic resistance and 16S rRNA profiles were demonstrated with alignment scores. *Salmonella* spp. prevalences according to regions were, 51 of 200 (25.50%) samples taken from Region A, 77 of 200 (38.50%) samples taken from Region B, 105 of 200 (52.50%) samples taken from Region C. Serotyping of the strains revealed that *S.* Typhimurium and *S.* Enteritidis are the most dormant strains among all strains. Antibiotic susceptibility of the strains revealed that major resistance against ampicillin. This study is held for an awareness rising about the possible impact of seasonality related with food borne pathogens prevalence.

Practical applications

Poultry meat and meat products account for approximately one-third of all *Salmonella* infections in humans. The relation between environmental temperature and foodborne pathogens is a complex matter, which has not been investigated widely and is hard to predict. The data obtained in this study indicate a significant high prevalence in warm region, which may be evaluated as a possible key for environmental temperature effect on foodborne pathogens distribution in organically reared poultry. In addition, this study provides important information to show the sources of contamination steps ranging from farm to fork in organically reared poultry.

1 | INTRODUCTION

The trend of organically grown foods has triggered a massive demand for these food products. Poultry products produced free range with less chemical residues are more demanded than integrated products. The term organic covers variable product groups due to the standards that differ worldwide. Organic farming in general features cultural, biological, and mechanical practices that foster cycling of resources, promote ecological balance, and conserve biodiversity. Synthetic pesticides and chemical fertilizers are not allowed in organic production, although certain approved pesticides may be used. In addition, organic foods are not allowed to use irradiation, industrial solvents, or synthetic food additives (Organization for Economic Co-operation and Development–OECD, 2015).

Fresh and processed poultry account approximately 29% of all *Salmonella* infections in humans (Braden, 2006). The most common serovars, which associated with human infections in the United States are *S.* Typhimurium, *S.* Enteritidis, *S.* Newport, *S.* Heidelberg, and *S.* Typhimurium-like serovars I 4,[5],12:i:- (Center for Disease Control and Prevention–CDC, 2008). Because of concerns with *Salmonella* in poultry, there have been a number of efforts to limit disease through different rearing/management practices, pre/probiotic use, antimicrobial therapy, and/or vaccination of birds against *Salmonella* and other pathogens. When data from nonconventional (organic, free-range, etc.) and conventional farms are compared, *Salmonella* prevalence was found to be dependent on the individual farm and not the farming system (Bailey & Cosby, 2005; Cui, Ge, Zheng, & Meng, 2005; Overbeke, Duchateau, De Zutter, Albers, & Ducatelle, 2006). As the demand

increases, the legislative concerns increased. In European Union, EC Reg. 1906/90 (European Commission–EC, 1990) (as amended by EC, 1993a; EC, 1993b) defines processing and marketing standards for poultry, including the optional use of indications concerning the type of farming (specifically: extensive indoor [barn-reared], free-range, traditional free-range and free range: total freedom). The detailed rules for farming types are introduced in EC Reg. 1538/91 (EC, 1991), which amends 1906/90. This latter regulation, which has itself been amended on several occasions (in particular by EC, 1993c; EC, 1994), covers terminology for different poultry species, part of birds, degree of evisceration, classification as Class A or B, conditions for freezing, chilling, pre-packing, water content and monitoring, as well as methods of production). Ministry of Agriculture, Fisheries and Food–MAFF (1996) gives an unofficial consolidated version of these changes.

Salmonella is an emerging pathogen that is isolated at high levels and not only effects flock health but also has a significant effect on public health. In a survey held in European Union, a 1 °C rise in global temperature was observed to trigger weekly rise of *Salmonella* incidence (Kovats et al., 2004). This study is designed to determine the prevalence of *Salmonella* isolated from poultry, which are reared in organic farms in three different ecological and geographical regions with distinct character. The aims are limited to the given but also antibiotic resistance and serotyping of strains were determined. These data will be the first to be reported in this geographical area.

2 | MATERIALS AND METHODS

This study is designed in three different organic poultry meat producers and slaughterhouses localized in three different regions between 34° and 36° longitudes of Turkey. These three regions show differences in seasonal and, vegetative characters, which are summarized below.

2.1 | Region A

Yozgat city exhibits continental climate, which is typical with the large temperature differences between summer and winter, and night and day. Temperature ranges between −23 and +37.1 °C through the year. Annually, approximately 40 days snowy and 20 days the temperature is below −10 °C. Annual precipitation averages 540 mm. Yozgat provincial land area of plantations and 56, 28% of the forest is composed of 15% meadows and pastures. The samples collected from Yozgat, Boz village, chicken breeders were located within the coordinates 39°47′30.4″N and 34°54′27.5″E. This area is mentioned as Region A. A sum of 80.000 chickens was reared during the sampling period.

2.2 | Region B

Samsun city exhibits temperate climate. Temperature ranges between −8.1 and +39 °C. Annually, number of days with recorded temperature is below 0 °C does not exceed 20 days. Annual precipitation averages 733 mm. Samsun typical has warm weather, abundant rainfall, and lush greenery. The samples collected from Samsun, Alaçam village, chicken breeders located within the coordinates 41°23′13.7″N and 35°55′25.3″E. This area is mentioned as Region B. A sum of 130.000 chicken was reared during the sampling period.

2.3 | Region C

Adana city exhibits Mediterranean climate. Temperature ranges between −8.4 and +45.6 °C. Annually, number of days with recorded temperature is below 0 °C does not exceed 5 days. Annual precipitation averages 980 mm. Adana typically has hot, humid weather. The samples collected from Adana, Deveciuşağı village, chicken breeders located within the coordinates 36°45′47.7″N and 35°37′13.8″E. This area is mentioned as Region C. A sum of 110.000 chicken was reared during the sampling period.

All samples were collected during October, January, April, and July. During 1 month sampling, 10 samples were taken from feed, from water bowls, and fecal swabs were collected. After poultry house samples were collected, the same flock was transferred to slaughterhouse where 10 neck skin samples and breast meat samples were collected. All samples were brought to the laboratory under cold chain conditions and analyzes were started within 6 hr.

2.4 | *Salmonella* isolation and identification

All food and feed samples were analyzed for *Salmonella* using the method ISO 6579:2002 (ISO, 2002) and fecal samples were analyzed using ISO 6579:2002, Amendment 2007. *Salmonella* strains were inoculated for motility using semi-solid Rappaport Vassiliadis agar (RVA, Oxoid, England) and incubated at 41.5 °C for 24 ± 3 hr (ISO, 2007). Motile *Salmonella* colonies are characterized by white/gray, turbid zones radiating from the point of inoculation. Zones surrounded by a white halo with a sharply defined borders were accepted as positive. All nonmotile *Salmonella* strains were omitted and they are not further analyzed. Motile *Salmonella* strains were confirmed using *inv*A specific PCR and strains were stored under −80 °C. PCR was applied using the method referred by Jeníková, Jarmila, and Katerina (2000).

2.5 | Serotyping

A service procurement was held from an internationally accredited commercial laboratory, which uses xMAP *Salmonella* Serotyping Assay (Luminex, Netherlands). All DNA were extracted and sent under cold chain using dry ice.

2.6 | Antibiotic susceptibility testing

Antibiotic susceptibility testing was held using the protocol reported by European Center for Disease Prevention and Control (ECDC) (2016) "draft protocol for surveillance of antimicrobial resistance of human isolates of *Salmonella* and *Campylobacter* EU harmonized". The antibiotic discs used in the measurement of sensitivity were as follows: ampicillin (CT0003B, Oxoid, England) from aminopenicillins group, ciprofloxacin (CT1615B, Oxoid, England) and nalidixic acid (CT0031B, Oxoid, England) from quinolones group, streptomycin

(CT1897B, Oxoid, England) and gentamicin (CT0794B, Oxoid, England) from aminoglycosides group, sulfamethoxazole (CT0074B, Oxoid, England) and trimethoprim (CT0076B, Oxoid, England) from sulfonamides group, chloramphenicol (CT0014B, Oxoid, England) from amphenicols group, tetracycline (CT0054B, Oxoid, England) from tetracyclines group, colistin (CT0664B, Oxoid, England) from polymyxins group, cefotaxime (CT0166B, Oxoid, England), ceftazidime (CT0412B, Oxoid, England), cefoxitin (CT0119B, Oxoid, England), cefepime (CT0771B, Oxoid, England) from cephalosporins group and lastly, meropenem (CT0774B, Oxoid, England) from carbapenem group. All zones were measured and antibiotic sensitivity was evaluated according to the table of European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2015).

2.7 | Extended-spectrum beta-lactamases disc screening

Disk-diffusion method for phenotypic extended-spectrum beta-lactamases (ESBL) screening was performed using cefpodoxime, ceftazidime, aztreonam, cefotaxime, and ceftriaxone, according to EUCAST and/or CLSI guidelines Table 1. As the affinity of ESBLs for different substrates is variable, the use of more than one of these agents for screening improves the sensitivity of detection. However, it is adequate to use the couple cefotaxime (or ceftriaxone) and ceftazidime. The inhibition zone around the cephalosporin disc combined with clavulanic acid is compared with the zone around the disc with the cephalosporin alone. The test is positive if the inhibition zone diameter is ≥5 mm larger with clavulanic acid than without.

2.8 | 16srRNA sequence analysis

After determining the antibiotic resistance, 10 strains, isolated from different sources from each region, were sent to a commercial laboratory for 16SrRNA sequence analysis. The sequences were analyzed using T-coffee multiple sequence alignment and dendograms were built (Notredame, Higgins, & Heringa, 2000).

TABLE 1 Serotype distribution of motile *Salmonella* strains isolated from different regions

Serotype distribution	Region A	Region B	Region C	Total
S. Hadar (6,8:z10:e,n,x)	1	0	5	6
S. Istanbul (8:z10:e,n,x)	0	1	0	1
S. Typhimurium ([1],4,[5], 12:i:1,2)	15	20	26	61
S. Enteritidis ([1],9,12:g,m:-)	11	13	16	40
S. Montevideo (6,7,[14]:g,m, [p],s:[1,2,7])	0	0	5	5
S. Infantis (6,7,[14]:r:1,5)	1	3	6	10
S. Mbandaka (6,7,[14]:z10:e, n,z15)	0	0	1	1
S. Jerusalem (6,7,[14]:z10:l,w)	1	0	1	2
S. Moers (11:m,t: -)	2	0	0	2
S. Dublin(1,9,12:[Vi]:g,p: -)	2	6	4	12
Total	**33**	**43**	**64**	**140**

2.9 | Seasonal distribution in sampling regions

Turkish State Meteorological Service's data were used to determine seasonal temperature distribution of sampling regions. The temperatures in Region A were normal in October 2013. On the contrary, the temperatures in Regions B and C were recorded to be lower than normal (a lower value between −0.98 and −2.51 °C) (Turkish State Meteorological Service–TSMS, 2014). The temperatures in all regions were recorded to be higher than normal (a higher value between 0.98 and 2.51 °C) in January 2014 (TSMS, 2015). The temperatures in Region B were normal in April 2014. On the contrary, the temperatures in Regions A and C were recorded to be higher than normal (higher between 0.98 and 1.69 °C). The temperatures in Regions B and C were recorded to be at normal values in June 2014. On the contrary, the temperatures in Region A were recorded to be higher than normal (higher between 0.98 and 1.99 °C) (TSMS, 2015).

3 | RESULTS AND DISCUSSION

3.1 | Distribution of *Salmonella* spp.

Although many factors including the hygienic practices are known to have an impact on prevalence of *Salmonella* spp., three different regions with different seasonal warmth and precipitation may have an effect on the prevalence recorded in this study. The results of the study showed that *Salmonella* were present in 233 of 600 (38.83%) samples. It was observed that 5 of 120 (4.16%) water samples, none of the 120 (0%) feed samples, 68 of 120 (56.66%) fecal samples, 95 of 120 (79.16%) neck skin samples, and 65 of 120 (54.16%) breast skin samples were contaminated with *Salmonella*. The data obtained were summarized in Figure 1.

This study was conducted in three different regions. The *Salmonella* spp. prevalences according to the regions were 51 of 200 (25.50%) samples taken from Region A, 77 of 200 (38.50%) samples taken from Region B, 105 of 200 (52.50%) samples taken from Region C. None of the 40 water samples, taken from Region A, were found to be contaminated with *Salmonella*. On the contrary, 2 of 40 (5%) from Region B and 3 of 40 (7.5%) from Region C were found to be contaminated with *Salmonella*. A sum of 11 (27.5%), 25 (62.50%), and 32 (80%) strains were isolated from 40 fecal samples from Regions A, B and C, respectively. In addition, 28 (70%) of 40 neck skin taken from slaughterhouse in Region A, 29 (72.50%) of 40 neck skin samples from Region B, and 38 (95%) of 40 neck skin samples from Region C were found to be positive for *Salmonella*. 12 (30%), 21 (52.50%), and 32 (80%) of the breast meat samples from Regions A, B, and C were found to be contaminated with *Salmonella*, respectively. The distribution of *Salmonella* in different regions, with respect to the samples, is summarized in Figure 2.

The relation between environmental changes and responses of foodborne pathogens is a complex matter, which has not been screened widely and is hard to predict (Miraglia et al., 2009). It is known that the increasing temperature along with the high precipitation rates is an additional value for survival and spread of virus, protozoa, and bacteria (James & James, 2010; Miraglia et al., 2009). In this study Region C, with its highest annual temperature, represented

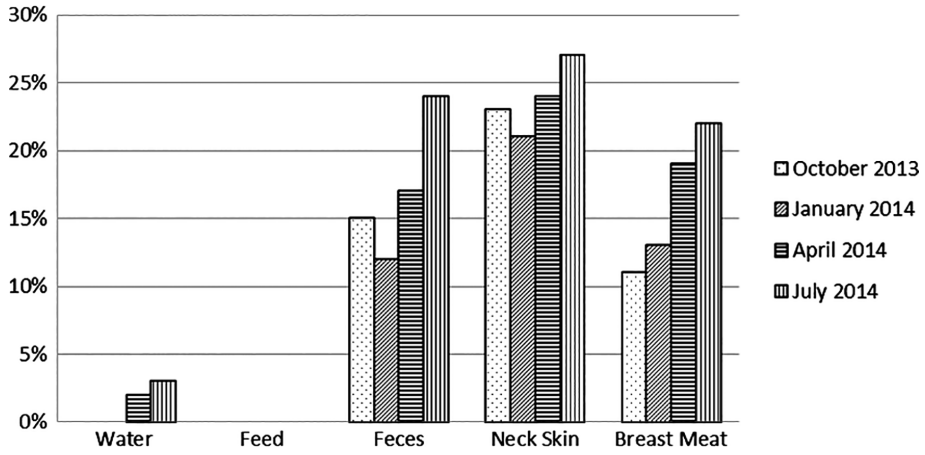

FIGURE 1 Seasonal distribution of *Salmonella* spp

Salmonella prevalence with 52.50%, which is followed by Region B with 38.50% and A with 25.50%. The increase in environmental temperature causes increase in vector mobility, which is reported for *Salmonella* and *Campylobacter* (Hall, D'souza, & Kirk, 2002; Pangloli et al., 2008). This situation explains the higher prevalence in warm Region A. In a study, Semenza and Menne (2009) demonstrated that if the multiplication base temperature accepted as 5 °C, each 1 °C increase in environmental temperature causes an increase of 5–10% in salmonellosis cases. Likewise, in Australia, D'souza, Becker, Hall, and Moodie (2004) reported this increase rate as 4–10%. Bi, Zhang, Hiller, and Cameron (2009) reported as 7% in Adelaide. Fleury, Charron, Holt, Allen, and Maarouf (2006) has reported as 1.2% for each 1 °C increase.

3.2 | Serotyping results

All isolates were confirmed with PCR and evaluated for motility. A sum of 233 strains was evaluated and 140 of them were found to be motile. Distribution of motile strains, according to the regions, was as follows: 33 motile strains from Region A, 43 motile strains from Region B, and 64 motile strains from Region C. Serotyping of the strains revealed that *S.* Typhimurium is the most dormant strain among all strains. *S.* Enteritidis was the second most dormant serotype. Other strains and their distribution are given in Table 1.

After serotyping of 140 strains isolated three most isolated strains were identified as *S.* Typhimurium (43.58%), which was followed by *S.* Enteritidis (28.57%) and *S.* Dublin (8.57%). Álvarez-Fernández, Alonso-Calleja, Camino García-Fernández, and Capıta (2012) showed that *S.* Enteritidis and *S.* Typhimurium are dormant strains, which were followed by *S.* Poona, *S.* Infantis, and *S.* Newport. Abd-Elghany, Sallam, Abd-Elkhalek, and Tamura (2015) reported the strain distribution in their study as *S.* Enteritidis (37.3%), *S.* Typhimurium (30.1%), *S.* Kentucky (10.8%), *S.* Muenster (8.4%), *S.* Virchow (4.8%), *S.* Anatum (4.8%), and *S.* Haifa (1.2%). On the contrary, Thai, Hirai, Lan, and Yamaguchi (2012) in their study held in North Vietnam, reported *S.* Emek (21.74%) as the dormant strain, which was followed by *S.* Infantis (13.04%), *S.* Blockey (12.17%), and *S.* Anatum (11.3%). Bacci et al. (2012) showed a distribution of 93 *S.* Enterica isolates from chicken carcass as 36 *S.* Virchow and followed with *S.* Derby (14%), *S.* Livingstone (11%), *S.* Saintpaul (8%), *S.* Enteritidis (8%), *S.* Hadar (6%), and *S.* Agona (3%). In a European study, public health hazard related strains were declared as *S.* Enteritidis, *S.* Typhimurium, *S.* Hadar, *S.* Virchow, *S.* Infantis, and *S.* Typhimurium (1,4,[5], 12:i:-) (EU, 2011; EFSA, 2011). In France, *S.* Typhimurium (46%), *S.* Enteritidis (19%),

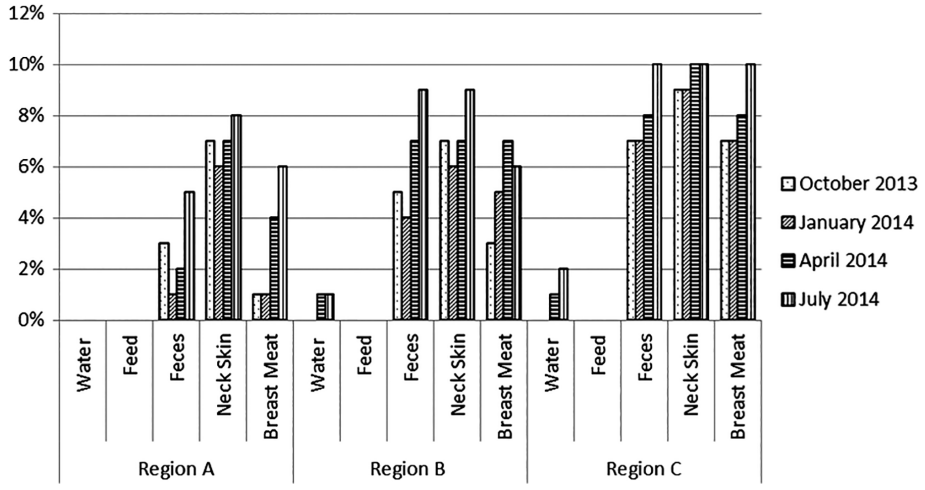

FIGURE 2 The distribution of *Salmonella* spp. in different regions with respect to samples

TABLE 2 The percentage (%) of multiple resistant strains according to regions

Distribution of antibiotic resistance (%)	Region A (*n* = 33)	Region B (*n* = 43)	Region C (*n* = 64)	Total (*n* = 140)
Ampicillin	42.42	37.21	45.31	42.14
Ciprofloxacin	3.03	11.63	25.00	15.71
Nalidixic acid	6.06	6.98	1.56	4.29
Streptomycin	12.12	9.30	7.81	9.29
Gentamicin	6.06	2.33	9.38	6.43
Sulphamethoxazole	57.58	41.86	37.50	43.57
Trimethoprim	42.42	20.93	32.81	31.43
Chloramphenicol	0.00	0.00	1.56	0.71
Tetracycline	9.09	4.65	14.06	10.00
Colistin	3.03	0.00	3.13	2.14
Cefotaxime	3.03	9.30	9.38	7.86
Ceftazidime	21.21	4.65	1.56	7.14
Cefoxitin	18.18	6.98	10.94	11.43
Cefepime	6.06	2.33	6.25	5.00
Meropenem	27.27	16.28	14.06	17.86

and *S.* 1,4,[5],12:i:- (4%) serovars were reported as most isolated strains (Switt, Soyer, Warnick, & Wiedmann, 2009).

This study is known to be the first report about antibiotic resistance, prevalence, and serotyping of organic poultry in Turkey. There is no publication or report about the subject of this study. On the contrary, antibiotic resistance of *Salmonella* has been a significant subject and data acquired are summarized in below. In a study, Kalender and Muz (1999) reported that 57 of 527 chicken samples in Elazıg were Salmonella positive. In addition, 39 were serotyped as *S.* Enteritidis and 14 as *S.* Gallinarum and 4 was as *S.* Typhimurium. In a study held in France, authors reported that widespread application of quinolones in veterinary practice triggered the resistance in *Salmonella* (Cailhol et al., 2006). The same was confirmed by Şahan et al., 2016 in a study in Turkey. In a study held by Yener, Akçelik, Şanlıbaba, and Akçelik (2012), *Salmonella* isolation was performed with 41 different samples. While the 25% of these 41 isolates were source of veal, the other 75% were source of chicken samples. At the same time, all of these tested 41 strains exhibited multidrug resistance profile. The highest resistance levels at all tested strains were determined against kanamycin (R > 512 μg/mL) and nalidixic acid (R > 512 μg/mL) for all strains.

3.3 | Antibiotic susceptibility

Antibiotic susceptibility of the strains revealed that major resistance against ampicillin. Strains isolated from samples taken from Region C were found to be more resistant to ampicillin, ciprofloxacin, gentamicin, chloramphenicol, tetracyclin than strains isolated from other two regions. On the contrary, strains isolated from samples taken from Region A were found to be more resistant to streptomycin, sulfamethoxazole, trimethoprim, ceftazidime, and meropenem. In addition, strains isolated from samples taken from Region B were found to be more resistant to nalidixic acid, cefotaxime than other strains isolated from other two regions. The percentage (%) of multiple resistant strains according to regions is summarized in Table 2. The numbers of multiple resistant *Salmonella* serotypes is given in Table 3.

ESBL screening test results are given in Table 4. ESBL positive strain from Region A was *S.* Typhimurium, which was isolated from feces in June 2014. The serotype of the ESBL (+) strain from Region B was *S.* Typhimurium, which was isolated from neck skin in April 2014. Lastly, the ESBL (+) strains from Region C were *S.* Typhimurium and *S.* Infantis, of which both were isolated from feces in June 2014.

In our study, 9 *S.* Typhimurium and 5 *S.* Enteritidis strain isolated from Region A were recorded to be resistant to less than four antibiotics, on the contrary, 6 *S.* Typhimurium and 6 *S.* Enteritidis strains were shown to be resistant more than four antibiotics. Likely, 5 *S.* Typhimurium and 4 *S.* Enteritidis strains isolated from Region B were resistant less than four antibiotics and reversely, 15 *S.* Typhimurium and 9 *S.* Enteritidis strains were resistant more than four antibiotics. Finally, the picture in Region C can be explained as, 14 *S.* Typhimurium and 6 *S.* Enteritidis strains isolated from Region B were resistant less than four antibiotics and reversely, 12 *S.* Typhimurium and 10 *S.* Enteritidis strains were resistant more than four antibiotics. A sum of all strains isolated in this study showed a distribution of 71 (50.71%) *Salmonella* strains isolated were resistant less than four antibiotics and 69 (49.29%) were resistant more than four antibiotics. Sapkota

TABLE 3 The numbers of multiple resistant *Salmonella* serotypes

	Region A		Region B		Region C	
	<4 Antibiotics	≥4 Antibiotics	<4 Antibiotics	≥4 Antibiotics	<4 Antibiotics	≥4 Antibiotics
S. Hadar	1	0	0	0	4	1
S. Istanbul	0	0	1	0	0	0
S. Typhimurium	9	6	5	15	14	12
S. Enteritidis	5	6	4	9	6	10
S. Montevideo	0	0	0	0	4	1
S. Infantis	1	0	2	1	5	1
S. Mbandaka	0	0	0	0	1	0
S. Jerusalem	1	0	0	0	1	0
S. Moers	2	0	0	0	0	0
S. Dublin	0	2	2	4	1	3
Total	**19**	**14**	**14**	**29**	**36**	**28**

TABLE 4 ESBL screening test results and distribution of the strains in different regions

Combination disc test (CDT)	Cefotaxime alone and with clavulanic acid	Ceftazidime alone and with clavulanic acid	ESBL (+) strain
Region A	3	2	1
Region B	6	1	1
Region C	9	4	2

et al. (2014) reported that 21% of *Salmonella* isolated were representing multiple resistance (six or more) to antibiotics. Álvarez-Fernández et al. (2012) showed that limiting antibiotic usage can trigger decrease in multiple resistance patterns. In our study, highest resistance was recorded to sulfamethoxazole with a rate of 43.57%, which is followed by 42.14% to ampicillin and 31.43% to trimethoprim.

In this study, strains showed a resistance mostly to sulfamethoxazole (43.57%) followed by ampicillin (42.14%) and trimethoprim (31.43%) (Table 2). Abd-Elghany et al. (2015) reported highest resistance to nalidixic acid (98.8%), sulfamethoxazole (96.4%), ampicillin (91.6%), and gentamicin (21.7%). Álvarez-Fernández et al. (2012) published that nalidixic acid, ampicillin, and chloramphenicol resistances as 100, 10.5, and 5.3%, respectively. In our study, nalidixic acid, gentamicin, and chloramphenicol resistances were found as 4.29, 6.43, and 0.71%, respectively. Different results obtained may be related with the management practices of slaughterhouses.

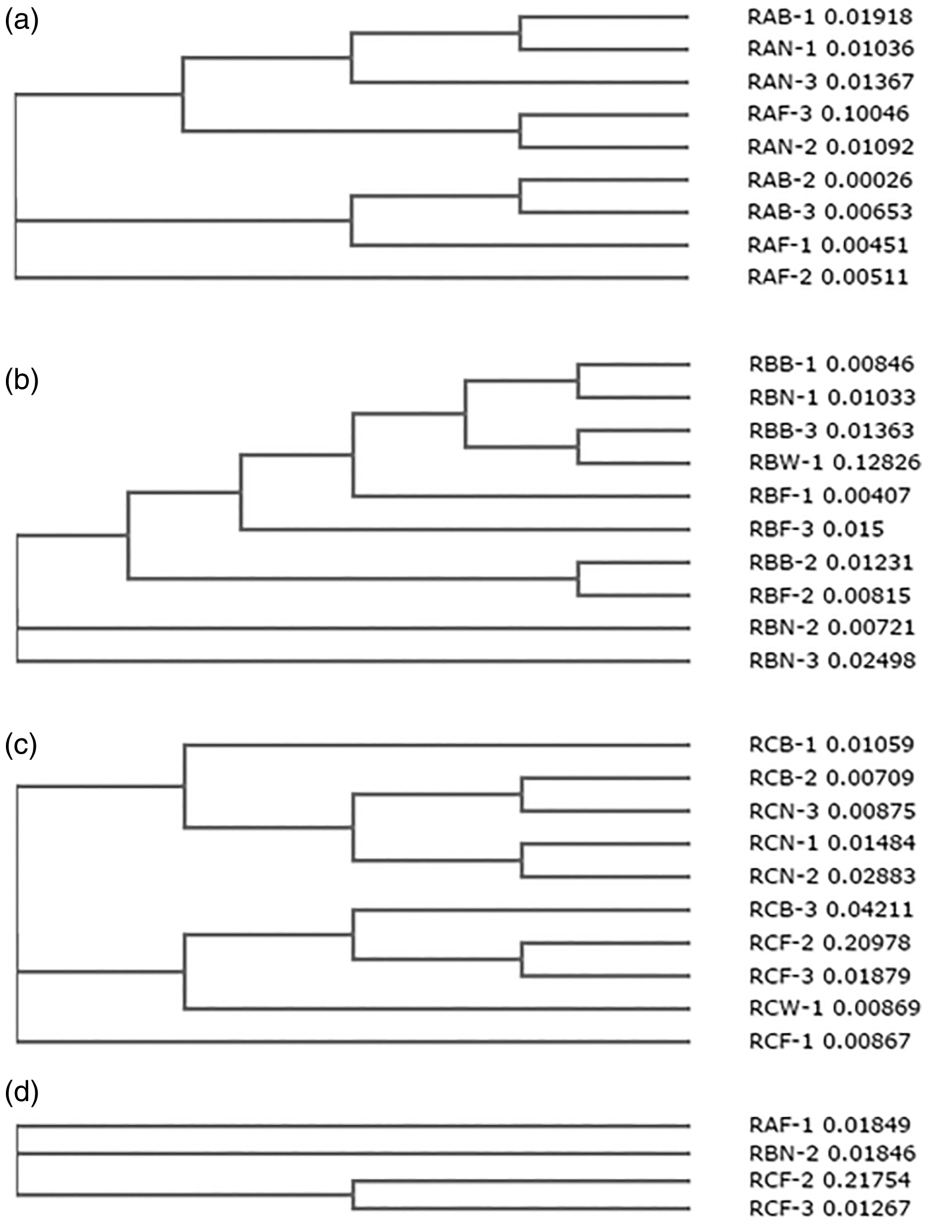

FIGURE 3 (a) Multiple sequence alignment results of Region A (RAB = breast sample from Region A; RAF = fecal sample from Region A; RAN = neck skin sample from Region A), (b) Multiple sequence alignment results of Region B (RBB = breast sample from Region B; RBF = fecal sample from Region B; RBN = neck skin sample from Region B; RBW = water sample from Region B), (c) Multiple sequence alignment results of Region C (RCB = breast sample from Region C; RCF = fecal sample from Region C; RCN = neck skin sample from Region C; RCW = water sample from Region C), (d) Multiple sequence alignment results of Region C (RAF = fecal sample from Region A; RBN = neck skin sample from Region B; RCF = fecal sample from Region C)

3.4 | Multiple sequence alignment results

Multiple sequence alignment results of Region A are shown in Figure 3a. The figure shows that RAB-1 and RAN-1, RAF-3 and RAN-2, and RAB-2 and RAB-3 are significantly similar to each other ($p < .05$). RAF-2 is simplicifolious and RAF-1 is the lesser similar strain to all strains. Multiple sequence alignment results of Region B are shown in Figure 3b. The figure shows that RBB-1 and RBN-1, RBB-3 and RBW-1, and RBB-2 and RBF-2 are significantly similar to each other ($p < .05$). RBN-2 and RBN-3 are simplicifolious and, which the lesser similar strains to all strains. It can be assumed that RBB-3 sample was contaminated with the waterborne strain RBW-1. Multiple sequence alignment results of Region B are shown in Figure 3c. The figure shows that RCB-2 and RCN-3, RCN-1 and RCN-2, and RCF-2 and RCF-3 are significantly similar to each other ($p < .05$). RCF-1 is simplicifolious and it is the least similar strain to all strains. Multiple sequence alignment results of ESBL (+) strains (Figure 3d) revealed a statistically significant similarity between RCF-2 and RCF-3. However, RAF-1 and RBN-2 were simplicifolious.

The multiple alignment of the strains by regions showed that the major contamination source of the chickens in Region A is feces, Region B is feces and Region C is water. It is shown that chicken can be contaminated with *Salmonella* with any stage of rearing period and feed, water and environmental contaminants play a major role. It is clear that fecal shedding and spread causes an increase in *Salmonella* prevalence in the facilities (Alali, Thakur, Berghaus, Martın, & Gebreyes, 2010). As the source of contamination varies, control of *Salmonella* contamination becomes harder and leads an increase in prevalence. In addition, increase in environmental temperature triggers increase in mobility in vectors and survival rates of pathogens (Figure 1). Moreover, slaughter process is important for contamination. In different stages, contamination occurs and leads an increase in prevalence of *Salmonella* (Abu-Ruwaida, Sawaya, Dashti, Murad, & Al-Othman, 1994; Göksoy, Kirkan, & Kök, 2004; González-Miret, Escudero-Gilete, & Heredia, 2006). As the data of this study indicate, *Salmonella* prevalence of neck skin (79.16%) samples are higher than fecal samples (56.66%), which underlines a cross contamination and this is proved by sequence analyzes data (Figure 3a–c).

4 | CONCLUSION

Our study data indicates that in warm regions, prevalence and antibiotic resistance of the strains are higher than cold regions. A long time screening study will be an additional value to determine the correlation between environmental temperature and *Salmonella* prevalence.

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