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Prevalence of *Salmonella* in poultry slaughterhouses located in Tripoli, Libya

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Abstract

Background: *Salmonella* is a leading cause of severe economic losses in poultry and foodborne illness in humans worldwide.

Aim: The aim of this study was to determine the prevalence and multidrug resistance of Salmonella Enteritidis (S. Enteritidis) in several chicken abattoirs in Tripoli, Libya. The study includes the South, East, and West regions of Tripoli.

Methods: Each region was assigned five slaughterhouses. Each chicken slaughterhouse was visited three times to collect samples. Five samples were taken at random from the neck skin, crop, and spleen. The total number of samples collected from all regions was 675. Bacterial isolation and identification, as well as antibiotic sensitivity testing, were performed on these samples.

Results: *Salmonella* spp. was found to be 15% prevalent, and S. Enteritidis was found to be 7% prevalent. The south region of Tripoli had the highest S. Enteritidis (9%), while the west region had the highest *Salmonella* spp. (22%). *Salmonella* prevalence increased significantly ($p < 0.01$) higher in the spleen (13%) as compared with the crop (5%) and neck (7%). Based on bacterial resistance pattern, *Salmonella* spp. isolated from the spleen had the highest multiple antibiotic resistance (MAR) index of 0.86 in the south region followed by MAR indexes of 0.8 and 0.46 in the West and East, respectively.

Conclusion: Isolation of *Salmonella* from the spleen may indicate chickens' systemic infection and failure to control the most important microbe for public health. Thus, the control measures have to be revised and a national *Salmonella* control program should be put in place urgently.

Keywords: *Salmonella* spp., *Salmonella* Enteritidis, Slaughterhouses, Broiler, Tripoli.

Introduction

Salmonella is a leading cause of severe economic losses in poultry and foodborne illness in humans worldwide (Lin *et al.*, 2014; Sallam *et al.*, 2014). Though there are more than 2,000 different subspecies of *Salmonella*, few can cause serious conditions in humans and chickens (Rodpai *et al.*, 2013). *Salmonella* Enteritidis (*S.* Enteritidis) is invasive in laying hens, and vertical transmission has been demonstrated (Cooper *et al.*, 1989). Some strains of *S.* Enteritidis have been shown to cause anorexia, diarrhea, and decreased egg production in experimentally infected laying hens (Gast and Beard, 1992). *Salmonella* Enteritidis is also invasive in broiler chickens and is frequently isolated from the muscles of raw chicken carcasses purchased from retail outlets (Lister, 1988; Humphrey, 2000). Infection caused by *Salmonella* rather than *S.* pullorum-gallinarum (typhoid fever), the condition is known as paratyphoid fever (PT), however, signs of severe PT infection in young poultry are commonly seen with *S.* pullorum-gallinarum gallinarum similar to things. The presence of *Salmonella* in the gut, skin, and feathers of chickens can contaminate carcasses during slaughter and processing, potentially contributing to the introduction of this organism into the slaughterhouse (Paiao *et al.*, 2013). *Salmonella* Enteritidis pollution is a major problem in many countries everywhere in the world. In 2018, there were 419 confirmed cases of *Salmonella* identical to raw chicken, including frozen breaded raw chicken products in Canada (BCCDC, 2018). Since 2014, human cases of *S.* Enteritidis in the European Union (EU) have increased by 3%. Over the same period, the prevalence of *Salmonella* in egg production increased from 0.7% to 1.21%. Salmonellosis was mentioned in 94,530 cases in the EU in 2016. *Salmonella* Enteritidis, the most frequent condition of *Salmonella*, accounts for 59% of all salmonellosis cases in the (EU) and is primarily associated with the consumption of eggs,

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egg products, and poultry meat (BCCDC, 2018). In Germany, 16,000 human cases of salmonellosis were reported. *Salmonella* Enteritidis was the most common serotype (44.4% of all isolates), as it had been in prior years (Antunes *et al.*, 2016). Expanding resistance to antibiotics in *S.* Enteritidis and other *Salmonella* spp. is a problem leading to serious health risks worldwide (Singh *et al.*, 2013). The reason for this problem could be due to the overuse and misuse of antibiotics in developing countries (Ikwap *et al.*, 2014). In Libya, there were few researchers studied the prevalence of *Salmonella* in chicken slaughterhouses as well as in humans. Serotyping of *Salmonella* isolated from children with diarrhea in Zliten City resulted in the presence of *S*. Heidelberg and *S*. Enteritidis isolates (Ali *et al.*, 2005). Therefore, the purpose of this research was to estimate the prevalence of *Salmonella* in poultry slaughterhouses in Tripoli, Libya's south, west, and east regions.

Material and Methods

Sampling

The survey covered the southern, eastern, and western regions of Tripoli during the 2018 period. From each region, five chickens were selected from each slaughterhouse. Each chicken abattoir was visited three times at 2-week intervals for sampling. Samples taken from each abattoir including neck skin, crops, and spleen were collected. The total number of samples from all regions was 675. All five samples were pooled prior to isolation and identification. Therefore, 135 samples were processed for isolation, identification, and antibiotic susceptibility testing.

Isolation and identification

The *Salmonella* isolation procedure was used as per the method of Waltman *et al.* (1993). The swabs were inoculated in pre-enrichment media (peptone water, Oxoid, Hampshire, UK) at 35°C–37°C for 24 hours. A loopful of the pre-enrichment medium was then inoculated in the selective-enrichment broth, Rappaport Vassiliadis (Oxoid, Hampshire, UK) at 35°C–37°C for 24 hours after collection. A loopful of the selective-enrichment broth was then streaked on the selective media, xylose lysine desoxycholate (Oxoid, Hampshire, United Kingdom) agar at 35°C–37°C for 24 hours. The morphology of the bacteria was tested by Gram stain. The isolates were then identified biochemically using a single colony selected and inoculated in triple sugar iron (Merck, Darmstadt, Germany) agar, citrate, lysine, indol, urea, and oxidase (Merck, Darmstadt, Germany). The positive colony was submitted to the National Centre for Animal Health for serotyping using slide agglutinations O1, O9, O12, and H including [f], g, m, and [p]antigens.

Antimicrobial sensitivity test (the Kirby-Bauer disc method)

The antibiotic susceptibility of the isolates was tested by using the disc diffusion method described by Bauer *et al.* (1966) with minor modifications. Fresh 3–5 colonies of the isolate were collected and suspended in 1 ml. sterile saline. The suspension was then standardized to a 0.5 McFarland standard (equivalent to approximately 1.5 × 108 CFU/ml). The prepared suspension was used as-inoculated within 15 minutes. The suspension was blotted with a sterile, non-toxic cotton swab, streaked onto a Mueller-Hinton agar plate (Merck, Darmstadt, Germany), and allowed to dry for 2–4 minutes. Antimicrobial susceptible discs (Oxoid, Hampshire, UK) were then placed in the cultures using a diffusion disc dispenser (Oxoid). Antibiotic discs tested were Ciprofloxacin, Trimethoprim, Chloramphenicol, Amoxicillin/Clavulanic acid, Sulfamethazone-Trimethoprim, Ampicillin, Gentamycin, Doxycyclin, Colistin, Neomycin, Tetracycline, Nitrofurantoin, Lincomycin, Erythromycin, and Cefuroxime. The zone of inhibition was assessed after 24 hours of incubation at 37°C to determine the degree of

Table 1. Number and percentage of *Salmonella* spp. and *S.* Enteritidis isolated from different organs and different regions.

Organs	*Salmonella* isolates						Total/135	
	West		South		East			
	***S.* spp.**	**SE**	***S.* spp.**	**SE**	***S.* spp.**	**SE**	***S.* spp.**	**SE**
Crop	3/15	0/15	1/15	1/15	0/15	0/15	4 (3%)	1 (1%) 5 (4%)[a]
Neck	2/15	2/15	2/15	0/15	0/15	1/15	4 (3%)	3 (2%) 7 (5%)[a]
Spleen	5/15	1/15	4/15	3/15	3/15	1/15	12 (9%)	5 (4%) 17 (13%)[b]
Percent	10/45 (22%)	3/45 (7%)	7/45 (16%)	4/45 (9%)	3/45 (7%)	2/45 (4%)	29/135 (21%)	
Total	13		11		5		29	

S. spp.: *Salmonella* species; SE: *Salmonella* Enteritidis. Data within a column lacking a common superscript differ at ($p < 0.01$)

sensitivity (sensitive, intermediate, or resistant). The multiple antibiotic resistance (MAR) indexes were determined using the formula: *a*/*b*, where "*a*" is the number of antibiotics to which a particular isolate was resistant and "*b*" is the total number of antibiotics tested (Krumperman, 1983).

Statistical analysis

Salmonella prevalence data were subjected to Pearson's chi-square test using Statistical Package for the Social Sciences software (SPSS Inc. Chicago, IL) to determine the significant variation, if any, among different regions (west, south, and east) and organs (crop, neck, and spleen). The value of ($p < 0.01$) was taken as the cut-off value to consider differences statistically significant.

Ethical approval

All work was done using international animal welfare standards. All the samples are collected from using the National Center of Animal Health (NCAH) protocol.

Table 2. Prevalence of *Salmonella* spp. and *S.* Enteritidis isolated from different regions of Tripoli.

Type of *Salmonella*	Number of *Salmonella* isolates			Total
	West	South	East	
Salmonella spp.	10/45 (22%)	7/45 (16%)	3/45 (7%)	20/135 (15%)
S. Enteritidis	3/45 (7%)	4/45 (9%)	2/45 (4%)	9/135 (7%)
Total	13/45 (29%)[a]	11/45 (24%)[a]	5/45 (11%)[a]	29/135 (21%)

[a] Within a row, data labeled with letters indicate no significant differences ($p > 0.05$).

Results

Prevalence of Salmonella

In the current study, *Salmonella* spp. and *S.* Enteritidis were isolated from 29 out of 135 samples collected from three regions of Tripoli (Table 1). In the West region, 13 (29%) out of 45 chicken organs collected from 5 slaughterhouses, were positive for *Salmonella*. Among those, 3 (7%) were positive for *S.* Enteritidis and 10 (22%) were positive for *Salmonella* spp. In the south region, a total of 11 (24%) out of 45 chicken organs collected from 5 slaughterhouses, were positive for *Salmonella*. Among those, 4 (9%) were positive for *S.* Enteritidis and 7 (16%) were positive for *Salmonella* spp.

In the East region, 5 (29%) out of 45 chicken organs collected from 5 slaughterhouses, were positive for *Salmonella*. Among those, 2 (4%) were positive for *E.* Enteritidis and 3 (7%) were positive for *Salmonella* spp.

The overall prevalence of Salmonella isolates from different regions was 21% (Table 2). The prevalence of S. Enteritidis was 7%. The highest prevalence of S. Enteritidis (9%) was recorded in the southern region of Tripoli. Salmonella has the highest prevalence (22%). However, it is found in the western regions. Statistically, there were no significant differences ($p > 0.05$) in the prevalence of total *Salmonella* between the regions. In general, the prevalence of *Salmonella* was significantly ($p > 0.01$) higher in the spleen (13%) as compared with crop and neck where the prevalence of *Salmonella* in these organs was 4% and 5%, respectively. In the spleen (9%) of isolated *Salmonella* were *Salmonella* spp. and only 4% were *S.* Enteritidis.

Antibiotic sensitivity test

The resistance pattern, *Salmonella* spp. isolated from the spleen had the highest MAR index value of 0.86 in the South region (Table 3) followed by a MAR index

Table 3. Antimicrobial resistance profiles of *S.* Enteritidis and *Salmonella* spp. isolated from chickens in the South region.

Isolate no.	*Salmonella* serovar	Organ	Antibiotic resistance profiles	MAR index
1	S. Enteritidis	Crop	AMC AM DO TE F MY E CXM	0.53
2	*Salmonella* spp.	Spleen	CIP TMP AMC SXT AM CN DO CT TE F MY E CXM	0.86
3	*Salmonella* spp.	Spleen	CIP TMP AMC SXT AM CN DO CT TE F MY E CXM	0.86
4	*Salmonella* spp.	Neck	CIP TMP AMC SXT AM CN DO CT TE F MY E CXM	0.86
5	S. Enteritidis	Spleen	AMC AM DO TE F MY E CXM	0.53
6	*Salmonella* spp.	Spleen	TMP SXT AM CN DO CT F MY E CXM	0.66
7	*Salmonella* spp.	Neck	CIP TMP AMC CXT AM CN DO CT F MY E CXM	0.8
8	S. Enteritidis	Spleen	AMC AM DO TE F MY E CXM	0.53
9	S. Enteritidis	Spleen	AMC AM DO TE F MY E CXM	0.53
10	*Salmonella* spp.	Crop	CIP TMP AMC SXT AM CN DO CT F MY E CXM	0.8
11	*Salmonella* spp.	Spleen	CIP TMP AMC SXT AM CN DO CT TE F MY E CXM	0.86

MAR index: the number of resistant antibiotics/the total number of antibiotics tested; CIP: Ciprofloxacin; TMP: Trimethoprim; AMC: Amoxycillin/clavulanic acid; SXT: Sulfamethazon trimethoprim; AM: Ampicillin; CN: Gentamycin; CT: Colistin; DO: Doxytetracyclin; TE: Tetracycline; MY: Lincomycin; E: Erythromycin; CXM: Cefuroxime; N: Neomycine; F: Nitrofurantoin.

Table 4. Antimicrobial resistance profiles of *S.* Enteritidis and *Salmonella* spp. isolated from chickens in the West region.

Isolate no.	Salmonella serovar	Organ	Antibiotic resistance profiles	MAR index
1	*Salmonella* spp.	Crop	AM CN MY E CXM	0.33
2	*Salmonella* spp.	Neck	CIP TMP AMC SXT AM CN DO TE F MY E CXM	0.8
3	*Salmonella* spp.	Spleen	CIP TMP AMC AM CN DO TE F MY E CXM	0.73
4	S. Enteritidis	Spleen	AMC AM MY E CXM	0.33
5	S. Enteritidis	Neck	AMC AM MY E CXM	0.33
6	*Salmonella* spp.	Spleen	CIP TMP AMC SXT AM CN DO TE F MY E CXM	0.8
7	*Salmonella* spp.	Spleen	CIP TMP AMC SXT AM CN DO TE F MY E CXM	0.8
8	*Salmonella* spp.	Crop	AM CN F MY E CXM	0.4
9	*Salmonella* spp.	Spleen	CIP TMP AMC SXT AM CN DO CT F MY E CXM	0.8
10	S. Enteritidis	Neck	AMC AM MY E CXM	0.33
11	*Salmonella* spp.	Spleen	CIP TMP AMC AM CN DO TE F MY E CXM	0.73
12	*Salmonella* spp.	Crop	AM CN F MY E CXM	0.4
13	*Salmonella* spp.	Spleen	CIP TMP AMC SXT AM CN DO CT F MY E CXM	0.8

MAR index: the number of resistant antibiotics /the total number of antibiotics tested; CIP: Ciprofloxacin; TMP: Trimethoprim; AMC: Amoxycillin/ clavulanic acid; SXT: Sulfamethazone trimethoprim; AM: Ampicillin; CN: Gentamycin; CT: Colistin; DO: Doxytetracyclin; TE: Tetracycline; MY: Lincomycin; E: Erythromycin; CXM: Cefuroxime; N: Neomycine; F: Nitrofurantoin.

Table 5. Antimicrobial resistance profiles of *S.* enteritidis and *Salmonella* spp. isolated from chickens in the East region.

Isolate no.	Salmonella serovar	Organ	Antibiotic resistance profiles	MAR index
1	*Salmonella* spp.	Spleen	AM CN DO TE MY E CXM	0.46
2	*Salmonella* spp.	Spleen	AM CN DO TE MY E CXM	0.46
3	S. Enteritidis	Neck	AMC AM MY E CXM	0.33
4	S. Enteritidis	Neck	AMC AM MY E CXM	0.33
5	*Salmonella* spp.	Spleen	AM CN MY E CXM	0.33

MAR index: the number of resistant antibiotics /the total number of antibiotics tested; CIP: Ciprofloxacin; TMP: Trimethoprim; AMC: Amoxycillin/ clavulanic acid; SXT: Sulfamethazon trimethoprim; AM: Ampicillin; CN: Gentamycin; DO: Doxytetracyclin; TE: Tetracycline; MY: Lincomycin; E: Erythromycin; CXM: Cefuroxime; N: Neomycin; F: Nitrofurantoin.

Table 6. Antimicrobial resistance pattern of *S.* enteritidis, and *Salmonella* spp. isolated from chicken organs samples tested by disc diffusion method (total of 29 isolates).

No.	Antimicrobial agent	Anti-bigram pattern of *Salmonella* Enteritidis and *S.* spp.		
		Resistant (%)	Intermediate (%)	Sensitive (%)
1	Ciprofloxacin	45	0	55
2	Trimethoprim	48	0	52
3	Chloramphenicol	0	52	48
4	Amoxycillin/clavanic acid	76	24	0
5	Sulfamethazon trimethoprim	41	0	59
6	Ampicillin	100	0	0
7	Gentamycin	69	0	31
8	Doxytetracyclin	69	17	14

Continued

No.	Antimicrobial agent	Anti-bigram pattern of *Salmonella* Enteritidis and *S.* spp.		
		Resistant (%)	Intermediate (%)	Sensitive (%)
9	Colistin	28	0	72
10	Neomycin	0	38	62
11	Tetracycline	52	20	28
12	Nitrofurantoin	69	17	14
13	Lincomycin	100	0	0
14	Erythromycin	100	0	0
15	Cefuroxime	100	0	0

value of 0.8 in the West region (Table 4) and a MAR index value of 0.46 in the East region (Table 5). The highest MAR index of *S.* Enteritidis isolated from the spleen and crop was 0.53 in the South region (Table 3). Ampicillin, Lincomycin, Erythromycin, and Cefuroxime resistance have been observed for all isolated bacteria. However, resistance to Colistin 8 (28%), and Sulfamethazon-trimethoprim 12 (41%), were found to be low. Colistin 21 (72%) had the highest sensitivity, followed by Neomycin 18 (62%), while Nitrofurantoin 4 (14%), and Doxy tetracycline 4 (14%), had the lowest (Table 6).

Discussion

In the current study, *Salmonella* spp. and *S.* Enteritidis were isolated from 29 out of 135 samples collected from three regions of Tripoli. The overall prevalence was 21%. The prevalence of *Salmonella* spp. was 15% whereas the prevalence of S. Enteritidis was 7%. This high prevalence might reflect poor hygienic and biosecurity measures in poultry houses, slaughterhouses, and live bird markets. Similar results were reported by Paiao *et al*. (2013) in Brazil and by Karim *et al*. (2017) in Bangladesh. A lower prevalence of *Salmonella* spp. (0.39%) and S. Enteritidis (1.18%) was reported in Poland (Witkowska *et al*., 2018). In Turkey, Goncag *et al*. (2005) reported a prevalence of 8.57% for S. Enteritidis in chicken carcass skins of the wing parts. In Algeria, Djeffal *et al*. (2018) also reported a prevalence of 8% for *Salmonella* spp. isolated from the skin of the chicken. However, Ramya *et al*. (2012) reported a higher incidence of *Salmonella* spp. and S. Enteritidis in chickens in India. They reported a prevalence of 64% (16 out of 25) and 56% (14 out of 25) for *Salmonella* spp. by polymerase chain reaction (PCR) and culture, respectively, and a prevalence of 48% (12 out of 25) for S. Enteritidis by PCR. Salmonellosis is a very important zoonotic disease in human beings causing diarrhea, nausea, abdominal pain, mild fever, chills, vomiting, prostration, headache, and malaise (Forshell and Wierup, 2006).

Among the regions included in this study, the southern region of Tripoli had the highest prevalence of S. Enteritidis (9%) and the west seemed to have the highest prevalence of *Salmonella* spp. (22%). These two regions are known for their intensive poultry production. Studies on the extent of biosecurity measures in chicken farms in Tripoli are lacking, but poor biosecurity measures in poultry farms may be one of the causes of the spread of Salmonella. In the study by Kammon *et al*. (2017) the levels of biosecurity in poultry farms located in Aljabal Al-Gharbi, particularly house floors, Farm distance, the existence of cleaning products at farm entrances, use of coveralls, disposal of dead birds, and low controls of birds and rodents. 63% of poultry houses have a ground of soil and 44% of them have uncoated walls which may influence the proper cleaning and disinfection. In the current study, while visiting the slaughterhouses for sampling, low levels of biosecurity measures were observed including the absence of regular use of disinfectants, absence of coverall cloths, the presence of multiple clots of blood on walls and ground, and dirty chicken feather removing machine and cutting knives. Moreover, some slaughterhouses are located nearly to the accumulation of municipal sewage just in front of the main door. Mostly there was no program to control the flies, wild birds, and rodents. Following proper sanitation and biosecurity procedures reduces the possibility of *Salmonella* contamination. Sanitizing water pipes, keeping wild birds and other animals out of slaughterhouses, limiting visitor numbers to required staff, using and maintaining footbaths on a regular basis, and wearing shoe covers or special shoes. Controlling insects and rodents are a common biosecurity practice in poultry farms (Dorea *et al*., 2010; Van Steenwinkel, 2011). In general, the prevalence of *Salmonella* was significantly ($p < 0.01$) higher in the spleen (13%) as compared with crop and neck where the prevalence of *Salmonella* in these organs was 4% and 5%, respectively. In the spleen, 12 (9%) isolated *Salmonella* spp., and only 5 (4%) were S. Enteritidis. The prevalence of *Salmonella* in the spleen was 47%, 40%, and 27% in the South, West, and East regions, respectively. This result may indicate systemic infection of chickens with *Salmonella*. Previously, Asheg *et al.* (2003) confirmed the presence of *S.* Enteritidis in macrophage-like cells, particularly in the lamina propria of the cecum, 3–21 days after infection. *S.* Enteritidis colonization and migration in the chicken

intestinal tract were found to be dose-dependent with the ability of macrophages to survive after phagosome/lysosome fusion (Oh *et al.*, 1996). *Salmonella* Enteritidis isolates have been linked to spleen invasion in various ways (Asheg *et al.* 2002). Our result is in contrast to previous studies in which the crop was implicated as an important agent of carcass contamination within the processing plant (Ramirez *et al.*, 1997). Hargis *et al.* (1995) found elevated levels of *Salmonella* in crops than in ceca during commercial evisceration. However, the localization of *Salmonella* in the gut, skin, and under feathers of chickens can contaminate carcasses during slaughter and processing, potentially contributing to the introduction of this organism into slaughterhouses (Paiao et al., 2013). Our study shows 100% resistance of *S.* Enteritidis and *Salmonella* spp. to ampicillin, lincomycin, erythromycin, and cefuroxime. The high and MAR indexes of 0.86 and 0.53 for *Salmonella* spp. and *S.* Enteritidis isolated from the spleen were found in the South region. Resistance to erythromycin has been reported as the most common resistance profile in retail meat production (Sallam *et al.*, 2014). Thung *et al.* (2016) have found 100% resistance of *Salmonella* to erythromycin, 69% to gentamycin, 100% to ampicillin, 45% to ciprofloxacin, and 52% to tetracycline. In another study, Bhuvaneswari *et al.* (2015) reported 60.7%, 92.1%, 100%, 23.5%, and 92.1% resistance of *Salmonella* to erythromycin, gentamycin, ampicillin, ciprofloxacin, and tetracycline in India, respectively. Antimicrobial resistance in *S.* Enteritidis and other *Salmonella* spp. is an increasing problem leading to serious health hazards in the world (Singh *et al.*, 2013). The reason for this problem could be due to the extensive use of antibiotics in developing countries (Ikwap *et al.*, 2014). In contrast, our study showed that isolated *S.* Enteritidis was susceptible to ciprofloxacin, trimethoprim, chloramphenicol, sulfamethazon trimethoprim, gentamycin, colistin, and neomycin. In a study by Thung *et al.* (2016), *S.* Enteritidis was susceptible to trimethoprim and gentamycin.

In conclusion, isolation of *Salmonella* from the spleen may indicate a chicken's systemic infection and failure to control the most important microbe for public health. Thus, the control measures have to be revised and a national *Salmonella* control program should be put in place urgently.

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Conflict of interest

The authors declare that there is no conflict of interest.

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