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Isolation of *Salmonella* spp. from Chicken Meats in Northern Iraq

Kuzey Irak'ta Üretilen Tavuk Etlerinde *Salmonella* spp. İzolasyonu

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ÖZET

Bu çalışmada, Duhok ve Erbil'deki lokal market ve kasaplardan temin edilen taze ve dondurulmuş tavuk etlerinde Salmonella yaygınlığı araştırılmıştır. Bu amaçla, 50 taze ve 50 dondurulmuş tavuk eti kültürel, biyokimyasal ve serolojik yöntemlerle incelenmiştir. Ayrıca, elde edilen Salmonella izolatlarının 13 farklı antibiyotiğe hassasiyetleri ölçülmüştür. Uygulan kültürel metotta, 100 örnekten 11'inde Salmonella kolonileri görülmüştür. Bunlardan 9 tanesinin taze (%18), 2 tanesinin de (%4) dondurulmuş tavuk etlerinden ya da illere göre, 7 tanesinin (%15.6) Erbil'den 4 tanesinin de Duhok'tan (%7.3) toplanan tavuk etlerinden ileri geldiği bulunmuştur. Salmonella şüphesi bulunan 11 kültür daha sonra biyokimyasal doğrulama testlere tabi tutulmuş ve geleneksel metotlarla yapılan testlerde 8'inin ve RapIDTM ONE sistemi ile yapılan testte ise 11'inin de Salmonella olduğu görülmüştür. Somatic (O) and flagellar (H) antiseralar ile yapılan serolojik testlerde, Salmonella şüphesi bulunan 11 kültür aglütinasyon-pozitif bulunmuştur. Irak Merkez Laboratuvarında yaptırılan serotip çalışmasında, 8 kültürde S. typhimurium, 3 kültürde ise S. anatum bulunmuştur. Her iki serotipinde 13 antibiyotikten sadece 4 tanesine (CT, C, CIP ve TE), S. anatum'un ayrıca K ve TMP antibiyotiklerine duyarlı olduğu görülmüştür.

Anahtar Kelimeler, Tavuk eti, *Salmonella*, izolasyon, RapIDTM ONE test, serolojik test, antibiyotik direçliliği

ABSTRACT

The present study was conducted to isolate and confirm Salmonella in fresh and frozen chicken meats collected in local markets and butcheries in Duhok and Arbil, Northern Iraq. In this respect, fifty fresh and fifty frozen chicken meats were analyzed by the cultural, biochemical and serological methods. The suspected cultures were later subjected to the antibiotic resistance test using 13 known antibiotics. The cultural method for isolation of Salmonella indicated that 11 agar plates (9 from fresh (18%) and 2 from frozen (4%)) were probably suspected with Salmonella. Seven suspected meat samples was collected in Arbil (15.6%) and 4 in Duhok (7.3%). The biochemical confirmation tests performed later confirmed that 8 of 11 suspected culture contained Salmonella. The RapIDTM ONE test for further confirmation showed that all 11 suspected cultures probably infected with Salmonella with two different species. Using somatic (O) and flagellar (H) antisera, it was observed agglutination of the suspected cultures with polyvalent O and H antisera, indicating the suspected cultures contained Salmonella. The cultures were serotyped by the Central Health Laboratory, and confirmed that 8 of them were infected with S. Typhimurium, and 3 of them with S. Anatum. Both serotypes were found to be sensitive only to CT, C, CIP and TE. S. anatum also exhibited sensitivity to K and TMP.

Keywords, Chicken meat, *Salmonella*, isolation, RapIDTM ONE test, serological test, antibiotic resistance

1. INTRODUCTION

Illnesses from food are one of the most important economic and health problems among industrial and non-industrial countries. In recent years, *Salmonella* has been one of the most common causes of food born disease (Busani et al., 2006). Salmonellosis is an infectious disease which often occurs through contaminated food, especially food products with an animal origin such as meat, chicken, egg, animal foods and sometimes vegetables in the food chain (Bouchrif et al., 2009). *Salmonella* lives in the intestinal track of humans and other animals, including birds. Poultry is the largest source of *Salmonella* for humans (Lynch et al., 2006).

Poultry meat is contaminated with *Salmonella* not only by infected poultry, but also by cross-contamination with feces, water, instruments and worker's hands during the slaughter process and handling. Chicken might thus provide the main transmission route of infection, especially with the increasing consumer demand for this food. Poultry and poultry products are frequently contaminated with *Salmonella* that can be transmitted to humans through the handling of raw poultry carcasses and

products, or through consumption of undercooked poultry meat (Bailey and Cosby, 2003; Kimura et al., 2004).

Salmonella species are recognized as very important food borne, waterborne organisms and the cause of a significant range of illnesses including food poisoning (gastroenteritis), typhoid (enteric fever), paratyphoid, bactereamia, septicemia with different variety of sequelae (Bell and Kyriakides, 2002). *Salmonella* spp. are estimated to cause more than 30% of all bacterial foodborne deaths (Mead et al., 1999).

Salmonella food poisoning is caused by the ingestion of food contaminated with significant numbers of non-host specific species or serotypes of the genus *Salmonella* (Jay et al., 2005). The most incriminated foods in outbreaks of human salmonellosis are those of animal origin (D' Aoust, 1991), which usually resulted from infected animals used in food production or from the contamination of the carcasses or the edible organs (Alemayehu et al., 2003). Outbreaks of *Salmonella* food poisoning are most commonly associated with meat, meat products, poultry meat or raw-egg dishes and less commonly with milk, cream, sea foods, salad vegetables and canned meat[l]. Food with a high fat content or a good buffering capacity may protect a relatively small dose of *Salmonella* during their passage through the acidic region of the stomach, and thus permitting a lower dose than the normal to initiate food poisoning. Examples of such foods, which have been associated with *Salmonella* food poisoning, are chocolate and cheddar cheese (Quinn et al., 2011).

The clinical picture of *Salmonella* food poisoning is that of acute gastroenteritis consisting of fever, nausea, vomiting, abdominal cramps, headache, diarrhea and dehydration which are typical symptoms and severity ranges from mild pain and little diarrhea to the extreme pain and the bloody severe diarrhea (CDC, 2001).

There is an increasing trend in production and consumption of meat, poultry meat and their products. This, of course, requires adequate control and inspection both during poultry rearing and in slaughterhouses, processing plants and shops. Consumers are also a link in the chain of food-borne human diseases, because of the way they store and cook meat, poultry meat, and their products (Kozacinski et al., 2006). Therefore, microbial safety of food is a significant concern of consumers and industries today. Because of the extensive use of antimicrobial agents in animal and human therapy, resistance became a public health concern (Sefton, 2002). Drug resistant *Salmonella* emerge in response to antimicrobial usage in humans and in food animals and selective pressure from the use of antimicrobials is a major driving force behind the emergence of resistance (WHO, 2013).
One of the studies in Spain reported high percentages of resistance of *Salmonella* isolates to sulfadiazine, neomycin, tetracycline and streptomycin, which might be the result of the use of antibiotics as a prophylaxis, growth promoter or treatment (Carraminana et al., 2004). In another study in Spain, 55 *S. abortusovis* strains isolated from Spanish ovine flocks were screened for resistance to 18 antimicrobial agents and reported that all strains showed full streptomycin and spectinomycin resistance. Although 23 out 55 strains were sulphamethoxazole resistant, only four of them also expressed resistance to trimethoprim/ sulphamethoxazole (Valdezate et al., 2007).

A study in Alberta, Canada indicated a high resistance of *Salmonella* isolates from food and food animals to ampicillin, streptomycin, sulfamethoxazole and tetracycline (Johnson et al., 2005). Over the past decade in Nepal, increasing antibiotic resistance in *S. enterica* has led to a shift in the antibiotics used against this organism from chloramphenicol and ampicillin to trimethoprim/sulfamethoxazole, fluoroquinolones and ceftriaxone, where only a 16-40% positive response to treatment has been achieved (Pokharel et al., 2006). In another study, of 380 *Salmonella* isolates from animal origin in the US, 82% of the isolates were resistant to at least one antimicrobial, and 70% to three or more antimicrobials. Resistance was most often observed to tetracycline, followed by streptomycin, sulfamethoxazole, ampicillin, chloramphenicol, kanamycin, amoxicillin/clavulanic acid, and ceftiofur (Zhao et al., 2007).

In USA, Kalchayanand et al., (2007) reported that 174 isolates from lamb carcasses were susceptible to all antibiotics tested, with only one isolate, from a pre-evisceration carcass, being multidrug-resistant (MDR) *Salmonella.* The MDR *Salmonella* was the serotype *typhimurium* and was resistant to amoxicillin–clavulonic acid, ampicillin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline.

In Van, Turkey Aksakal et al., (2009) found that 9 *S. saintpaul* strains isolated from sheep were susceptible to ampicillin, enrofloxacin, tetracycline, oxytetracycline, gentamicin, neomycin, chloramphenicol, spectinomycin, cephalothin, amoxicillin/clavulanic acid, trimethoprim/ sulfamethoxzole and nalidixic acid and they were resistant to erythromycin and novobiocin, and two of the strains from rectal isolates were resistant to penicillin G. However Davies et al., (2004) in Great Britain reported that *Salmonella* strains isolated from feces of animals slaughtered in abattoirs were sensitive to amoxicillin/clavulanic acid, ampicillin, chloramphenicol, gentamicin, neomycin, trimethoprim/sulfamethoxzole and nalidixic acid. Molla et al., (2006) reported that *Salmonella* strains isolated from feces of sheep slaughtered in abattoirs in Ethiopia were susceptible to gentamicin, neomycin and nalidixic acid, whereas they were resistant to amoxicillin/clavulanic acid, ampicillin, cephalothin, chloramphenicol, trimethoprim/sulfamethoxzole, spectinomycin and tetracycline. Intorre et al., (2005) in Italy found that *Salmonella* strains isolated from sheep were resistant to penicillin and erythromycin.
In Iraq, Hadad and Jemel (1990) tested 122 *Salmonella* isolates of animal origin for antimicrobial susceptibility, only 15.6% of the isolates were sensitive to all of the antibiotics used, while 84.4% of the isolates were resistant to one or more of the antibiotics tested, most frequently they were resistant to sulphafurazol, tetracycline, streptomycin and chloramphenicol, however all isolates were sensitive to ampicillin, carbnicillin, gentamycin, furazolidone, cephaloridine and nalidixic acid. Similarly, Hadad and Ali

(1995) in Mosul, Iraq, reported that 72 *Salmonella* strains isolated from dairy products were resistant to tetracycline and sulphamethizole, while all isolates were sensitive to co-trimoxazole. Fifty-two isolates were resistant to three or more antibiotics and included resistant to tetracycline, sulphamethizole and streptomycin. Al-Abidy and Hadad (2007) in Mosul, Iraq, revealed that 35 *Salmonella* strains isolated from frozen poultry products were totally resistant to erythromycin and 32 isolates were resistant to 4 antibiotics (gentamicin, tetracycline, amoxicillin and nalidixic acid). Six patterns of multiple antibiotic resistant were found the most frequent resistance was to gentamicin, erythromycin, amoxicillin, tetracycline and nalidixic acid.

The purpose of the study was to establish the prevalence of *Salmonella* and to check its resistance to common antibiotics in chicken meats obtained from Northern Iraq.

2. MATERIALS AND METHODS

2.1. Materials

A total of 100 chicken carcasses were collected from local markets and butcheries during June 2013 to January 2014 in Northern Iraq. The meat samples used in the study were chosen either fresh or frozen (Table 1).

Table1. Collection areas of chicken samples

Source	Fresh	Frozen	No. of Sample
Duhok	25	30	55
Arbil	25	20	45
Total	50	50	100

Buffered peptone water, Rappaport-Vassiliadis (RV) enrichment broth, ABC chromogenic agar, Xylose Lysine Deoxycholate (XLD) agar, Nutrient agar, Kligler iron (KI) agar, Simmons citrate (SC) agar, Brilliant Green (BG) agar, Urease broth, Mueller Hinton (MH) agar and Brain heart infusion (BHI) broth were purchased from LabM, UK. Antibiotic Discs used in the study were purchased from Bioanalyse, Turkey (Table 2). RapID^{TM} ONE was purchased from Remel Inc (USA). *Salmonella* somatic (O) and flagellar (H) antisera were obtained from Plasmatec, UK.

Table 2. Antibiotics used in the study

	Antibiotic	Abbreviation	Concentration (µg/disc)
1	Amoxicillin/Clavulanic	AM	30
2	Carbenicillin	CT	25
3	Chloramphenicol	C	10
4	Ciprofloxacin	CIP	10
5	Doxycycline	DOC	10
6	Enrofloxacin	ENR	15
7	Erythromycin	E	15
8	Gentamicin	GN	30
9	Kanamycin	K	30
10	Nalidixic acid	NA	30
11	Nitrofurantoin	F	30
12	Tetracycline	TE	30
13	Trimethoprim	TMP	10

2.2. Sampling of Chicken Meats

Fresh carcasses were broken into pieces under hygienic conditions in the laboratory. The pieces from neck, wings, breast and drumstick were placed in sterile sample-collection bags, properly labeled and stored in a refrigerator or subjected to immediate analysis.

Frozen carcasses stored at freezer temperature for no longer than a week were thawed at the room temperature and broken into pieces under hygienic conditions in the laboratory. The pieces from neck, wings, breast and drumstick were placed in sterile sample-collection bags, properly labeled and stored in a refrigerator or subjected to immediate analysis.

2.3 Isolation

Representative meat samples were taken from neck, wings, breast and drumstick and grounded. Twenty-five grams of the meat sample were homogenized in 225 mL of peptone water using a stomacher (Bagmixer, Interscience) and incubated aerobically at 37±1°C for 18±2 hours. A volume of 0.1 mL homogenate was added into 10 mL of RV broth, vortexed and incubated aerobically at 41.5±2 °C for 24±3 hours for the selection of *Salmonella.* One loop (3-4 colonies) of the broth culture was plated on a XLD agar and one loop on an ABC agar for the subcultivated of *Salmonella*, and both were incubated aerobically at 37±1°C for 24±3 hours. Three suspected colonies having red color with black centers on the XLD agar and green color on the ABC agar were picked up and transferred separately on nutrient agar plates and streaked for increasing the number of *Salmonella.* Nutrient agar plates were incubated at 37±1°C for 24±3 hours. The colonies grown on the nutrient agar plates were later taken into biochemical and serological confirmation tests.

2.4. Biochemical Confirmation

The following biochemical tests were carried out on the suspected colonies according to the methods described by Quinn et al., (2011) and Brown (2004).

Glucose and lactose fermentation and hydrogen sulfide production in Kligler iron agar, It was applied for differentiation of microorganisms on the basis of glucose and lactose fermentation and hydrogen sulfide production (H_2S). Three-four colonies from the nutrient agar plates were stabbed into the butt and streaked the entire slant of a KI agar tube by using the sterile loop and incubated at 37±1°C for 24 ±3hours. Typical S*almonella* colonies in the KI agar tube were observed to have alkaline (red) slants and acid (yellow) butts (indicating only glucose fermentation; glucose-positive), to form bubbles from glucose fermentation and black precipitates from the hydrogen sulfide formation. However, the agar slant was yellow when *Salmonella* isolated fermented lactose (lactose-positive). Thus, the suspected *Salmonella* colonies were subjected to further tests for the confirmation.

Citrate utilization in Simmons citrate agar, It was applied to detect the ability of a microorganism to utilize the citrate as the sole carbon and energy source for growth and an ammonium salt as the sole source of nitrogen. A slant of SC agar tube was inoculated by 3-4 suspected *Salmonella* colonies isolated from the nutrient agar plates, and incubated at 37±1°C for 24±3 hours. Positive reactions were indicated by the growth of *Salmonella* on the SC agar tubes with the change in the color of agar from green to intense blue.

Urea utilization in Urease broth, This test is used to determine the ability of a microorganism to hydrolyze urea to form ammonia by action of the urease enzyme. *Salmonella* is always urease-negative. The suspected *Salmonella* colonies (3-4 colonies) from the nutrient agar were incubated on a urease broth tube at 37±1°C for 24±3 hours. While a urease-positive culture produced an alkaline reaction in the medium, evidenced by pinkish-red colour of the medium, urease-negative organisms did not change the color of the medium, which was pale yellow-pink.

RapID^{TM} ONE test, The suspected *Salmonella* colonies (3-4 colonies) from the nutrient agar plates were subcultured on a BG agar and incubated at 37±1°C for 24±3 hours. After incubation, 3-4 colonies were picked up and transferred into 2 mL of the RapID^{TM} ONE inoculation fluid (6 g KCl, 0.5 g $CaCl_2$, 1000 mL demineralized water) to achieve an inoculum with visual turbidity equal to a #2 McFarland turbidity standard. The suspension was vortexed and transferred into the upper right-hand corner of the panel of RapID^{TM} ONE system by using pipette. The panel was backed away from the reaction cavities at a 45° angle and racked gently side to side to evenly distribute the inoculum along rear baffles. The panel was slowly tilted forward toward the reaction cavities until the inoculum follows along the baffles into the reaction cavities. The inoculated panel was incubated at 37±1°C for 4 hours. After 4 hours incubation, the lid over of the reaction cavities was pulled off and then 2 drops of RapID^{TM} ONE reagent was added to cavities 15 (PRO) through 17 (PYR). Then cavities 1 (URE) through 18 (ADON) were scored and recorded in the report form by using test codes. Then 2 drops of RapID^{TM} ONE spot indole reagent was added to cavity 18 (ADON/IND), and was allowed for 10 seconds for color development and the score of cavity 18 (IND) was recorded in the report form by using the test code. The code numbers for (+) reaction in each triplet of biochemical reactions were added together. This gave a 7-digit (profile number) for the bacterium. The genus name was obtained by entering the 7-digit profile number into the computerized program (software) provided with RapID^{TM} ONE kit.

2.5. Serological Confirmation

Three separate drops of normal saline (0.85% sodium chloride) were placed on a clean glass slide. The suspected *Salmonella* colonies from the nutrient agar were added on two saline drops and mixed thoroughly on the slide to obtain a smooth suspension. The third drop on the slide was used as a control. One drop of somatic (O) and flagellar (H) antisera was added separately on to two drops of bacterial suspension on the slide in order to obtain a manner that each isolate was tested for agglutination against both antisera at the same time. Both antisera were mixed with bacterial suspensions by sterile loops. Then the slide was gently tilted back and forth for one minute. During this period agglutinations were observed under a normal lightening condition against a black background. All positive isolates were subcultured onto the slants of KI agar tubes, and submitted to the Central Health laboratory, Ministry of Health, Baghdad for serotyping.

2.6. Antibiotic Sensitivity Test

Kirby-Bauer disc diffusion method (Bauer et al., 1996) was used to determine the *in vitro* antibiotic susceptibility of the identified *Salmonella* isolates to various antibiotics given in Table 2. Antimicrobial susceptibility screening was conducted

using a panel of 13 agents. The concentrations of the antibiotics tested were 10-30 μg. A standardized suspension of the isolated *Salmonella* from the KI agar was adjusted to a 0.5 MacFarland turbidity standards and diluted 1,10 for the dilution method. A sterile swab was dipped into the standardized inoculum and used to inoculate evenly the surface of already prepared MH agar. The agar was left for 15 minutes for the surface moisture to dry. A multichannel disc dispenser (Oxoid, Basingstoke, UK) was used to deposit the antibiotics discs onto the surface of the inoculated medium. The plate was then incubated at 37°C for 24 h. The zones of growth inhibition were measured with slipping calipers. The diameter was read across the center of the discs and the results were interpreted according to the manufactures instructions.

3. RESULTS

The study was designed with the estimation of chicken meat prevalence of *Salmonella* as a central goal. One hundred fresh or frozen chicken meats from local markets and butcheries in Northern Iraq were sampled in order to provide a reasonably precise and unbiased estimate of *Salmonella* prevalence.

3.1. Isolation of Salmonella

Suspected *Salmonella* spp. were observed as red colonies with a black center on the selective XLD and green color on ABC agars. Eleven out of 100 meat samples were found to have susceptible *Salmonella* spp. The fresh meat samples (9) had higher prevalence of *Salmonella* spp. than the frozen meat samples (2). Arbil (15.6%) was observed to have 2 times more prevalence of *Salmonella* spp. than Duhok (7.3%). The prevalence of *Salmonella* spp in chicken meats is given in Table 3.

Table 3. The distribution of susceptible *Salmonella* spp. on chicken meat samples detected on XLD and ABC agars

Area	**Sample**	**No. of samples**	**Susceptible *Salmonella* spp. plates**	
			Number	**%**
Duhok	Fresh	25	3	12.0
	Frozen	30	1	3.3
Arbil	Fresh	25	6	24.0
	Frozen	20	1	5.0
	Total	**100**	**11**	**11.0**

3.2. Biochemical confirmation of Salmonella with Conventional Methods

The eleven suspected agar plates were subjected to the confirmation using the KI agar, SC agar and urease broth. Typical *Salmonella* cultures in the KI agar tubes were shown to form red (alkali) slant, yellow (acid) butt, black precipitates and gas bubbles. This indicated that *Salmonella* ssp. fermented glucose but not lactose and reduced sulfur compounds to H_2S. However, in some KI agar tubes, yellow (acid) slants were also observed, indicating formation of lactose by some *Salmonella* spp. In the SC agar tube, the color turned from green to intense blue in the presence of *Salmonella* spp. Since *Salmonella* is urease-negative, no color changed occurred in the urease broth tubes. The number of the *Salmonella* suspected tubes was 8 out of 11 suspected colonies (Table 4). Six of 9 fresh meat samples and all frozen meat sample (2) were found to be infected with *Salmonella* ssp. after the confirmation tests. The prevalence of *Salmonella* in the meat samples was more common in Arbil than in Duhok.

Table 4. The distribution of *Salmonella* spp. on chicken meats confirmed on KI broth, SC agar and Urease broth

Area	**Sample**	**No. of samples**	**Susceptible *Salmonella* spp.**	
			Number	**%**
Duhok	Fresh	3	2	66.7
	Frozen	1	1	100.0
Arbil	Fresh	6	4	66.7
	Frozen	1	1	100.0
	Total	**11**	**8**	**72.7**

3.3. Biochemical confirmation of Salmonella with RapIDTM ONE System

The suspected 11 agar plates were later subjected to the RapID™ ONE test for the confirmation by the conventional methods. The change in the color of the baffles was observed after the suspected *Salmonella* colonies were incubated.

All plates were suspected to contain S*almonella*, indicating that RapID™ ONE test is more sensitive than the conventional methods for the confirmation of *Salmonella* ssp. The results of the RapID™ ONE test are given in Table 5. Two different results were obtained from the RapID™ ONE test, (1) Positive results were found in the ODC, LDC, TET and SBL baffles, the even-digit number obtained was 4320000, and the data analysis program said 93% *Salmonella* spp. (2) Positive results were found in the ADH, ODC, LDC, SBL and GGT baffles, the even-digit number obtained was 6120010, and the data analysis program said 100% *Salmonella* spp.

Table 5. The result of the RapID™ ONE system

No	Test code	Reactive ingredient	Results	
1	URE	Urea	-	-
2	ADH	Arginine	-	+
3	ODC	Ornithine	+	+
4	LDC	Lysine	+	+
5	TET	Aliphatic thiol	+	-
6	LIP	Fatty acid ester	-	-
7	KSF	Sugar aldehyde	-	-
8	SBL	Sorbitol	+	+
9	GUR	nitrophenyle-□, D glucuronide	-	-
10	ONPG	nitrophenyl-□, D-galactoside	-	-
11	□□GLU	nitrophenyl-□, D-glucoside	-	-
12	□□XYL	nitrophenyl-□, D-xyloside	-	-
13	NAG	nitrophenyl-n-acetyl-□, D-glucosaminide	-	-
14	MAL	Malonate	-	-
15	PRO	Proline-□-naphthylamide	-	-
16	GGT	Glutamyl-□-naphthylamide	-	+
17	PYR	Pyrrolidonyl-□-naphthylamide	-	-
18	IND	Tryptophane	-	-

3.4. Serological Confirmation of Salmonella

The results of the serological test showed that the isolates gave positive reactions (agglutination) with polyvalent O and H antisera. The suspected 11 cultures were serotyped by the Central Health Laboratory in Baghdad and identified as *S. typhimurium* (8) and *S. anatum* (3). These two *Salmonella* ssp. were determined in the RapID™ ONE test as two different digit numbers, 4320000 probably indicates *S. typhimurium* and 6120010 indicates *S. anatum.*

3.5. Antibiotic Susceptibility of Salmonella

Antibiotic resistance of *S. typhimurium* and *S. anatum* was tested for susceptibility with 13 kinds of antibiotics by the disk diffusion method in MH agar. Table 6 shows the result of antibiotic sensitivity of *Salmonella* spp. Both *Salmonella* spp. were sensitive to CT, C, CIP and TE. *S. anatum* also showed sensitivity to K and TMP.

Table 6. Antibiotic sensitivity of *Salmonella* spp.

	Antibiotic	Abbreviation	Resistance	
			S. typhimurium	***S. anatum***
1	Amoxicillin/Clavulanic	AM	R	R
2	Carbenicillin	CT	S	S
3	Chloramphenicol	C	S	S
4	Ciprofloxacin	CIP	S	S
5	Doxycycline	DOC	R	R
6	Enrofloxacin	ENR	R	R
7	Erythromycin	E	R	R
8	Gentamicin	GN	R	R
9	Kanamycin	K	R	S
10	Nalidixic acid	NA	R	R
11	Nitrofurantoin	F	R	R
12	Tetracycline	TE	S	S
13	Trimethoprim	TMP	R	S

S, Sensitive; R, Resistant

3.6. Seasonal Variation in Salmonella Prevalence

There was significant seasonal variation in *Salmonella* isolates. High prevalence was recorded in the summer (13.3%), and low in the winter (4%) (Table 7).

Table 7. Seasonal variation in *Salmonella* prevalence

	No. of samples	**No. of isolate**	**% of isolate**
Summer	75	10	13.3
Winter	25	1	4

4. DISCUSSION

While it is widely acknowledged that *Salmonella* is the major cause of food borne illness in poultry and its products, epidemiological data are needed to inform public health authorities about the nature and size of the problem and to monitor trends over time. Raw poultry products are contaminated with harmful, pathogenic and spoilage bacteria by infected stocks, cross contamination, improper handling and storage or improper cooking of poultry, which can lead to human foodborne illness and loss of product shelf life (Myint, 2004). Food safety regulations on meat and poultry are important issues and there should be a global plan to address hazards by perfection the food safety at the animal production and intermediate stages before the slaughter plant, food safety during transportation, storage and retail sale, and to educate consumers to follow safe food handling practices such as proper storage, preparation, and cooking of meat and poultry products. However, in this study, there is a lack of knowledge at the animal production stages, of strategies followed by the exporting firm to reduce *Salmonella* in the live poultry, of slaughter technology and the level of food safety practiced by the industry.

This is the first study on the prevalence and antimicrobial resistance of *Salmonella* in fresh and frozen chicken carcasses sold in Duhok and Arbil, Northern Iraq. It is found an overall *Salmonella* prevalence of 11%. This rate is close to those reported by Al-Abidy and Hadad (2007) in Mosul, Iraq (9.72%), Ozbey and Ertas (2006) in Turkey (12%), Dahal (2007) in Bhutan (13%) and Maharjan et al., (2006) in Nepal (14.5%). However, the low prevalence of *Salmonella* in chicken carcasses was found compared to many other countries such as South Africa 19.2% (Van Nierop et al., 2005), Bangladesh 26.6% (Akond et al., 2012), Spain 35% (Dominguez et al., 2002), Vietnam 53% Van et al., 2007). The difference in the rates may be due to the fact that the actual prevalence of *Salmonella* is low or the processing industry decontaminate the final products or the producers use antimicrobials in the production chain or may be due to the isolation technique and number of samples examined.

Caution should be taken when making direct comparison between prevalence estimates of the pathogenic bacteria because of the differences in methods, samples number, frequency and time of sampling, transport and storage of samples, and season (Nouichi and Hamdi, 2009). The difference in the finding of the current study to the previous studies may be due to the difference in the number of samples examined and the methods used for the processing of samples. Among serotypes observed in this study, *S. typhimurium* (8) was the most common followed by *S. anatum* (3).

Approaches to prevent and control salmonellosis in the food animal industry by various means such as improved biosecurity, vaccination, use of competitive exclusion products, and the introduction of novel immuno-potentiators with limited success has necessitated the use of antimicrobial chemotherapy in the treatment and control of Salmonellosis (Zhao et al., 2007). The use of antimicrobials in food animals has resulted in the development of antimicrobial resistance (White et al., 2001), through mutation and acquisition of resistance encoding genes (Fluit, 2005). The situation in Iraq may be exaggerated by easy accessibility of antimicrobials at a cheaper price and their extensive use in poultry production. Another major setback might be the quality and potency of locally produced antimicrobial drugs. Thus there is widespread availability and uncontrolled use of antibiotics.

The result of antimicrobial susceptibility in this study showed that *S. typhimurium* (8 isolates) was sensitive to CT, C, CIP and TE, and *S. anatum* (3 isolates) to C CT, C, CIP, TE, K and TMP. This could be due to wide and varied use of antibiotic as treatment and feed additive in poultry. Excessive and inappropriate use of antibiotic in the rearing of farm animals of food chain represents a major factor in the emergence, persistence and spread of multi drugs resistant *Salmonella* (Cruchaga et al., 2001). These finding were concurrent to the observation in Iraq of Hadad and Jemel (1990), Al-Aboudi et al., (1993), Al-Abidy and Hadad (2007) and Abdulrahman (2010), and in other countries of Duffy et al. (1999), Dahal (2007), Smith et al. (2006) and Kaushik et al., (2014). All studies showed that *Salmonella* strains isolated from food and meat product of animal origin were multi drugs resistant.

There was a major variation in seasonal prevalence of *Salmonella* in summer and early winter months. A total of 75 samples were analyzed during the summer and 25 samples during the winter. High prevalence were was recorded in summer (13.3%), and low in winter (4%). The rate of contamination probably increase in high temperatures; the transportation and poor storage also play an important role in the prevalence of *Salmonella* during the summers in the Northern Iraq. The consignment is transferred and further transported in conventional vans. The temperatures rise as high as 38°C during summer in north Iraq.

This might be due to the fact that prevalence of *Salmonella* is higher during hot and dry seasons. These finding were similar to those reported in Nepal by Maharjan et al., (2006). They found that the prevalence of *Salmonella* was found the highest during the months of April and May, in a study in Bhutan, The isolation of *Salmonella* during winter and late spring was significantly different ($p<0.001$) (Dahal, 2007). Guran et al., (2017) reported that the overall *Salmonella* prevalence in the skin of skin-on chicken parts and meat of skin-off parts was 42.2% and 17.6%, respectively. In addition, have it was determined the overall *Salmonella* presence on whole chicken carcasses and chicken parts in many previous studies (Cook et al., 2012; Fearnley et al., 2011; Mazengia et al., 2014; Pointon et al., 2008; USDA, 2017a; Wu et al., 2014). The USDA-Food Safety and Inspection Service reported that *Salmonella* prevalence on young broilers was 3.8% based on the whole carcass rinse sampling method for Salmonella detection (USDA, 2017b). it was reported *Salmonella* prevalence on raw chicken parts with skin-on as 38.7% (USDA, 2017c). Moreover, it was found that *Salmonella* was present in 17.6% of 1300 retail chicken meat parts (breast, wing, and thigh samples with skin-on and skin-off) in a report (FDA), 2012). *Salmonella* prevalence in some chicken organ parts was in range of 12 - 9.9% in another study (Mazengia et al., 2014).

5. CONCLUSION

The present study was designed to isolate and confirm *Salmonella* in chicken meats collected in local markets and butcheries in Duhok and Arbil, Northern Iraq. In this respect, one-hundred fresh (50) and frozen (50) chicken meats were analyzed by the cultural, biochemical and serological methods. The suspected cultures were later subjected to the antibiotic resistance test using 13 known antibiotics.

The cultural method for isolation of *Salmonella* including pre-enrichment on buffered peptone water, enrichment in RV broth and selective enrichment in XLD and ABC agars indicated that 11 agar plates were probably suspected with *Salmonella*. The biochemical confirmation tests performed on the KI agar, SC agar and urease broth confirmed that 8 of 11 suspected culture contained *Salmonella*. The RapID^{TM} ONE test was further used for the confirmation. The RapID^{TM} ONE test result showed that all 11 suspected cultures probably contaminated with *Salmonella*. Two different digit numbers (4320000 and 6120010) obtained from the RapIDT^{M} ONE computer program indicated that the suspected cultures contained two different *Salmonella* spp.

In order to confirm *Salmonella*, the suspected cultures were subjected to the serological tests. Using somatic (O) and flagellar (H) antisera, it was observed agglutination of the suspected cultures with polyvalent O and H antisera, indicating the suspected cultures contained *Salmonella*. The cultures were serotyped by the Central Health Laboratory, and confirmed that 8 of them were contaminated with *S. Typhimurium*, and 3 of them with *S. anatum*.

S. typhimurium, and *S. anatum* were found to be sensitive only to CT, C, CIP and TE. *S. anatum* also exhibited sensitivity to K and TMP. This result indicated that *Salmonella* in the analyzed meat samples showed multi-drug resistance.

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