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Occurrence and antimicrobial resistance of Salmonella serotypes isolated from chicken carcasses in Duhok, Kurdistan Region / Iraq

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Abstract

This study was undertaken to evaluate the contamination rate of Salmonella serotypes and to determine their resistance profiling to six antibiotics from imported chicken carcass surfaces at the food control unit in the Duhok city / Ministry of Health, Kurdistan region of Iraq. in which the total 110 swabs were collected from April to June 2014. 21 samples out of 110 (19%) chickens were found to be contaminated with Salmonella and belonging to 4 different serotypes. S. ohio 13 (61.9%) was the most frequent serotype followed by S.newport 2 (9.5%), S.typhimurium 2 (9.5%), S.livingstone 1 (4.7%) and 3 (14.2%) isolates were non-typeable. 23.8% of the isolates were resistant to ciprofloxacin, 100% to cephalothin, 85.7% to trimethoprim-sulfamethoxazole, 28.5% to gentamycin, 76.1% ticarcillin-calvulanic acid while none of the isolates were resistant to imipenem (100% were susceptible). All Salmonella serotypes were resistant to at least one antibiotic and 16 (76.1%) have been resistant to three or more antibiotics. The results of this study indicate that there are high contamination rate of Salmonella in chicken carcasses with a high drug resistance profiling and require good strategies for controlling the pathogen during slaughtering. In addition, the selection of antibiotics should be with a more prudent

I. *Introduction*

Salmonella is the most common cause of food borne infections, and the second most common food borne illness second to *Campylobacter* infection (1). Which in turn regard as a major public health concern and responsibility for the significant cost in all around the world because millions of people are reported for *Salmonella* infection every year, which leads to the thousands of deaths (2, 3, 4). In recent years, there is an evidence for the increase in the consumption of poultry meat and its products in comparison with the other types of meat which intern preference by consumers due to their lower price (5). *Salmonella* infection outbreaks are usually related to the consumption of contaminated food such as eggs, poultry meat and pork (6).

The indiscriminate use of sub-therapeutic concentration of synthetic antimicrobial as growth promoters, therapeutics for prophylactic purposes provides the selection and prevalence of different serotypes of

Salmonella and other microorganisms resistant to antibiotics, leading to the reduction of their effectiveness in clinical cases, in addition to their residues in meat and their products (7, 5).
This study was undertaken to determine the presence, serotypes involved and characterize the levels of resistance to a variety of antibiotics in *Salmonella* serotype isolates from imported chicken carcass surface, in Duhok, Kurdistan Region of Iraq

II. *Materials and Methods*

A. *Sample collection*

The sampling process was carried out by swabbing on whole chicken Carcasses surface, starting from leg to neck. Total 110 swabs collected from randomly selected chicken carcasses that were imported from many countries at the food control unit in Duhok city Kurdistan Region of Iraq from April to June 2014. All swabs samples inserted in to sterile tubes containing 10 ml of buffered peptone water (BPW) (Lab M, UK) and immediately transported to the microbiology laboratory, Faculty of Veterinary Medicine University of Duhok and microbiological analyses carried out with in half hour after samples collection.

B. *Isolation and serotype identification*

Each swab in a test tube with (BPW) at delivery to the laboratory was mixed with vortex mixer then incubated at 37 ºC for 24 hours as pre-enrichment step. After that, 1 ml was transferred to 9 ml of Rappaport-Vassiliadis (RV) broth (Oxoid, UK) as selective enrichment and incubated at 42 ºC for 24 hours. After incubation, two loopful from RV broth were quadrant streaked on to xylose lysine deoxycholate (XLD) agars and incubated at 37 ºC for 24 hours (8, 9). Then suspected colonies of *Salmonella* spp., (red with black center) from XLD agar were identified by biochemical tests such as triple sugar iron agar (TSI), citrate utilization test, urease test (8) and miniaturized multi-test system RapID panel (Remmel, USA). The final confirmation was done by PCR technique using primers which are used for the detection specific sequence of *16s rRNA* gene ribosomal genes of *Salmonella* spp. (10). The identified *Salmonella* isolates were inoculated on to the TSI agar slants and submitted for serotyping in the national *Salmonella* serotyping center, Ministry of Health, Bagdad, Iraq.

C. *DNA extraction*

The DNA was extracted from biochemically identified *Salmonella* isolates by boiling method (11) with minor modification, briefly the isolates were inoculated in to 5ml of brain heart infusion (BHI) broth and incubated aerobically at 37 ºC for 24 hours. After incubation 1ml of each broth culture was transferred to a 1.5 ml micro-centrifuge tube and centrifuged for 10 min at 14,000 x g. Then the pellet was re-suspended in 300 µl of DNase / RNase free distilled water and centrifuged for 5 minutes. The pellet was again re-suspended in 200 µl of DNase / RNase free distilled water and incubated for 15 min at 100ºC then immediately chilled on ice for 3 min and again centrifuged for 5 min at 4 ºC. Finally, aliquot of 10 µl of the supernatant was used as DNA template for PCR (11).

D. *PCR Amplification*

Amplification was conducted in a total volume of 25 µL. The reaction mixture contained 12.5 ul of KAPA2G Fast ReadyMix master mix from (BIOSYSTEMS; USA) and consisted of 1.25 U Taq-Pol, 75 mM Tris-HCL (pH 8.8), 1.5 mM MgCl2, and 0.2 mM of each dNTP. The reaction mixture contained 12.5 ul master mix, 10 pmol of each forward and reverse primers ,2.0 µl of bacterial genomic DNA with concentration 200-250 ng/µl (measured by Nano drop Spectrophotometer), and 8.5 uL RNase free water to a total volume of 25 uL.
The condition for PCR that applied (11) detailed in table (1). After that, the PCR products observed by running on 2% agarose gel electrophoresis having 1.0 µg/ml ethidium bromide. In addition, the oligonucleotide primers Sal201-f CGGGCCTCTTGCCATCAGGTG and Sal597-r

CACATCCGACTTGACAGACCG were applied to amplified 16s rRNA gene, which used for specific detection of *Salmonella* spp. (11). The predicted size of amplified PCR product is 396 bp. The extracted DNA from *Salmonella Typhimurium* was used as the positive control was supported kindly by (Duhok Research Canter) and DNase free dH_2O was used as negative controls.

Table (1). Setting for PCR amplification of 16s rRNA gene primers.

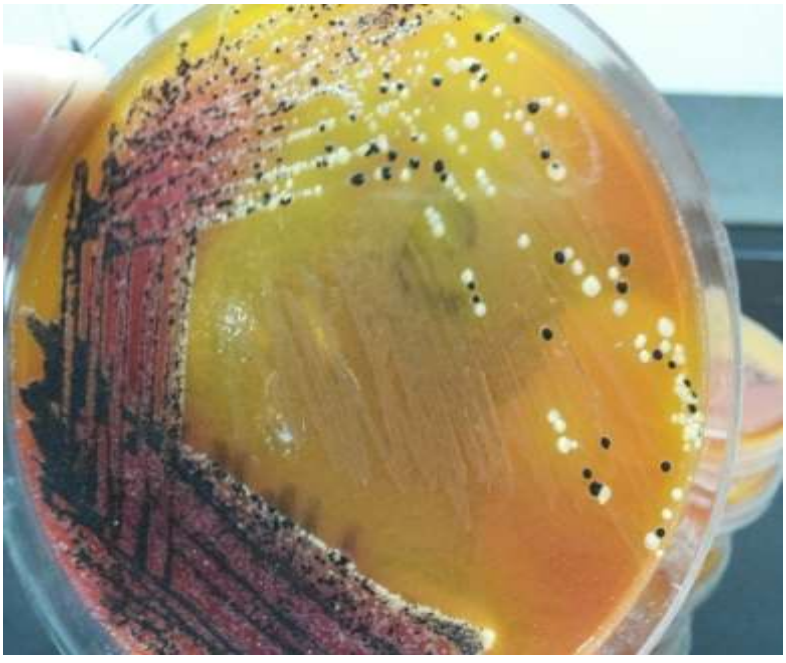
Step	Number of cycles	Temperature (°C)	Time
Pre denaturation	1X	95	3.0 min
Denaturation	35X	95	30 sec
Annealing		52	30 sec
Extension		72	90 sec
Final Extension	1X	72	5.0 min

E. *Resistant profiling determination*

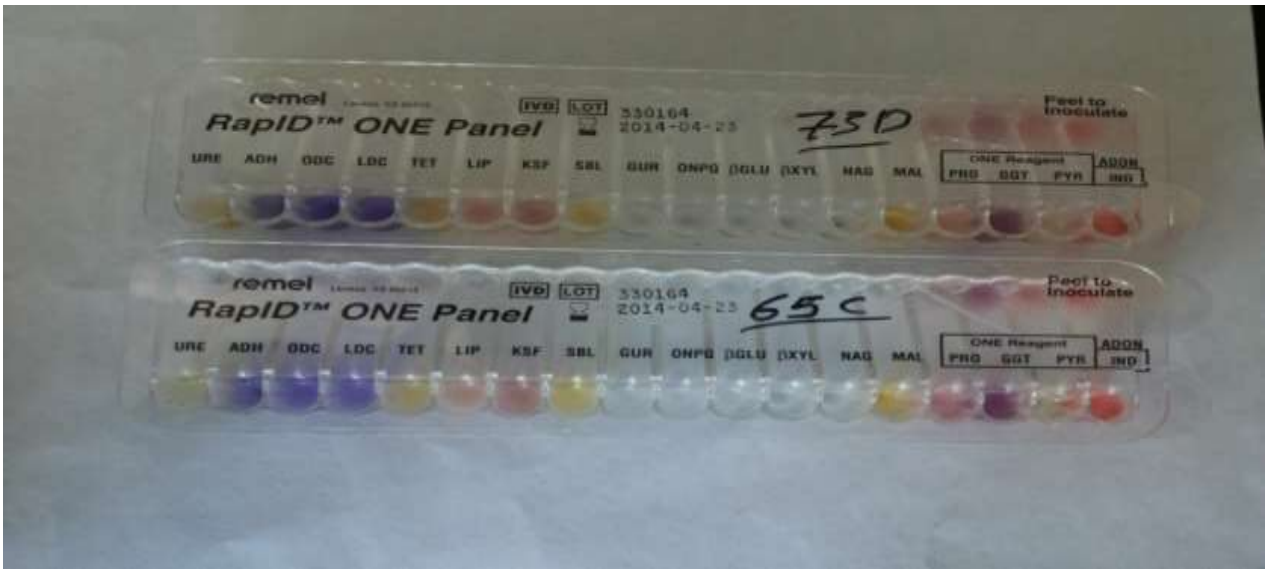
Antibiotic susceptibility testing was performed by a disc diffusion method on Mueller-Hinton agar according to (8) and the results were interpreted in accordance with Clinical and Laboratory Standards Institute (12). The serotypes were screened for their resistance to the following antibiotics from Bioanalyze Turkey: ciprofloxacin (CIP) 5 mcg, gentamycin (CN) 10 mcg, Imipenem (IM) 10 mcg, cephalothin (KF) 30 mcg, Trimethoprim-Sulfamethoxazole (SXT) 1.25-23.75 mcg, Ticarcillin-calvulanic acid (TCC) 75/10 mcg.

III. *Results*

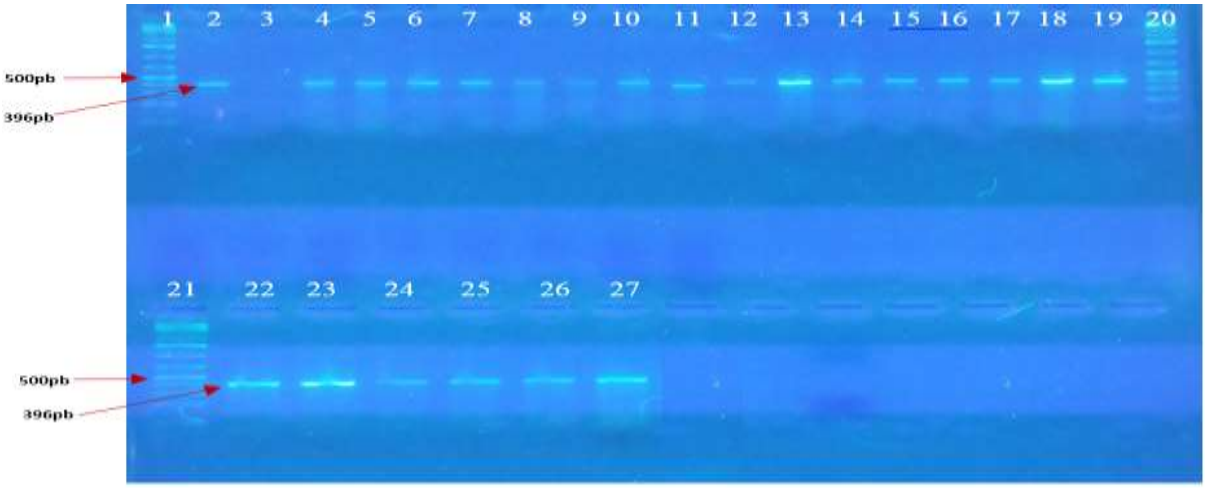
According to cultural (Fig. 1) biochemical characteristics (Fig. 2) and PCR assay (Fig. 3), the 110 samples (swabs) collected from imported chicken carcasses surfaces from various countries, only 21 (19%) were contaminated with *Salmonella* spp. Among the 21 *Salmonella* isolates, (4) different serotypes were identified; however the serotype could not be determined for three isolates. Among the serotypes, *S. ohio* 13 (61.9%) was the most frequent followed by *S.newport* 2 (9.5%), *S.typhimurium* 2 (9.5%), *S.livingstone* 1 (4.7%) and 3 (14.2%) isolates were non-typeable.



(Fig. 1): Typical red with black centered colonies of *Salmonella* spp., on XLD agar from primary isolation.

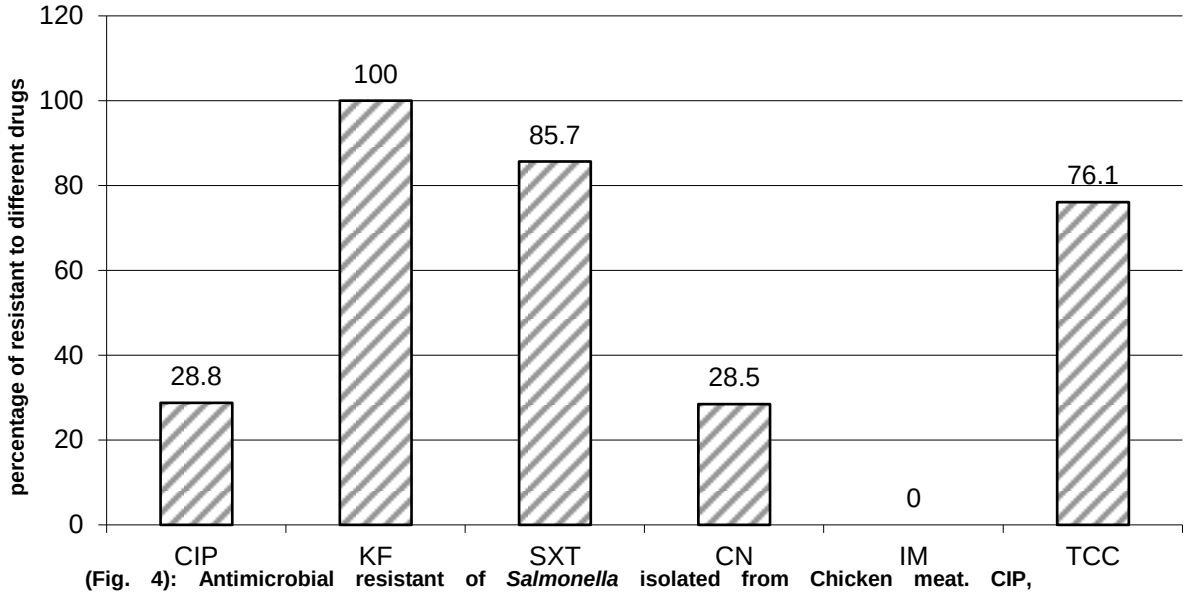


(Fig. 2): Characteristic biochemical reactions of *Salmonella* isolates with RapID One panel (Remmel, USA).



(Fig. 3) Amplification of 16s rRNA of *Salmonella* species on 2% Agarose gel showed under UV light. Lines 1, 20 and 2 SM1331Marker SM1331(Fermentans, Germany) , lines 2 and 22 Positive control, line 3 Negative control, lines 4-19 samples (41, 83, 20, 13, 17, 12, 80, 38, 84, 106, 108, 78, 93, 99, 48 and 102), lines 23-27 samples (5, 75, 23, 65 and 73).

From the 21 isolates, 23.8% of isolates were resistant to ciprofloxacin, 100% were resistant to cephalothin, 85.7% resistant to trimethoprim-sulfamethoxazole, 28.5% resistant to gentamycin, 76.1% to ticarcillin-calvulanic acid while none of the isolates were resistant to imipenem (100% were susceptible) (Fig. 4). It was observed that *S.ohio* and non-typeable *Salmonella* isolates revealed the highest percent of resistant to antibiotics tested followed by *S. typhimurium*, *S. newport* and *S.livingstone*. All *Salmonella* serotypes were resistant to at least one antibiotic and out of the 21 *Salmonella* isolates 16 (76.1%) showed resistant to tree or more antibiotics and were considered a multiple drug resistant (MDR), the most common MDR profile were KF, SXT and TCC (Table. 2).



(Fig. 4): Antimicrobial resistant of *Salmonella* isolated from Chicken meat. CIP, ceprofloxacin; KF, Cephalothin; SXT, Trimethoprim Sulphamethoxazol; CN, Gentamicin; IM, Imipenem; TCC, Ticarcillin calvolanic acid.

Table (2). Multiple antibiotic resistant pattern of *Salmonella* serotypes.

Isolates No., with serotypes	Antibiotic resistant No.	MDR pattern	No. (%) of isolates
S.ohio (08,102,23, 83 and 38)	4	KF, SXT, CN, TCC	5 (23.8)
S. ohio (0,65,84 and 106)	3	KF, SXT, TCC	4 (19)
S.ohio (12)	3	CIP, KF, TCC	1 (4.7)
S.ohio (75)	4	CIP, KF,SXT, TCC	1 (4.7)
S.ohio (13)	2	KF, SXT	1(4.7)
S.ohio (17)	2	KF, TCC	1 (4.7)
Non-typeable (99)	4	CIP, KF,,SXT, TCC	1 (4.7)
Non-typeable (78)	4	KF, SXT, CN, TCC	1 (4.7)
Non-typeable (48)	3	CIP, KF,,SXT	1 (4.7)
S.typhimurium(93)	3	KF, SXT, TCC	1 (4.7)
S.typhimurium(20)	2	KF, SXT	1 (4.7)
S.newport (5)	3	CIP, KF,,SXT	1 (4.7)
S.newport (73)	2	KF, SXT	1 (4.7)
S.livingstone (41)	2	KF, TCC	1 (4.7)

CIP:Ciprofloxacin, KF: Cephalothin, SXT: Trimethoprim-Sulfamethoxazole, CN: Gentamicin, TCC: Ticarcillin-clavulanic acid, MDR: Multiple Drug Resistant.

IV. *Discussion*

According to phenotypic characteristic, which is the main method for detection of bacteria, antibiotic sensitivity test and serology typing can be gained (13). However, in some cases, PCR technique required for specific detection of *Salmonella* spp., particularly when atypical culture characteristics appear (13). Therefore, PCR analysis with conventional cultural methods applied in this study to increase the detection specifity of *Salmonella*.

The percentage of *Salmonella* contamination on chicken carcasses in this study was (19%), which was lower than reported by Dallal *et al*., (14) and Dallal *et al*.(15) Were arrived (45%) and (62.7%), respectively, Also lower than (22%) by Saeed *et al*.(16) in Iraq, (44%) by Abd El-Aziz, (17) in Egypt, (37%) by Sakaridis *et al*.(5) in northern Greece and (44%) from conventional chickens by Cui *et al*.(18) in USA. Although it was greater than (5%) from post-grading point (post packaging) by Wang *et al*.(19) in China and (17.9%) by Capita *et al*.,(20) in Spain. There is a large difference in the *Salmonella* isolation rate from chicken meat between different studies; this may be attributed to the differences in sample number, methods of sample collection and the countries in which the *Salmonella* were isolated.

The high percentage of *Salmonella* contamination from chicken meat found in this study may be due to the high *Salmonella* shedding from the gastrointestinal tract of chickens that can contaminate the carcasses and the processing line during slaughter (21). Also, this may result from the contamination occur in the original slaughterhouse due to some factors that related to slaughtering process such as water used for washing, chilling and handling procedures (22) or during distribution. In addition, the high level of contamination indicates the presence of public health threatening. The level of risk will be higher if frozen chickens are not properly washed to remove the *Salmonella* biofilm found on their surfaces, consumed undercooked, cross-contamination occurs between raw and cooked chicken's food, or any other foods, during meal preparation in the kitchen or contamination of food in the retail shops.

In our study, we found out four *Salmonella* serotypes, *S.ohio, S.livingstone, S.newport* and *S.typhimurium*. S.*ohio* was the most common serotypes, this may be resulted in contamination happened during processing in the slaughterhouse or reflect that this serotype was usually present in the intestinal tracts of chickens contaminating the carcasses during the slaughtering process.

S.livingstone was isolated for the first time in Iraq during the last decades and for the first time in Kurdistan region/Iraq. However, other serotypes have been isolated from chickens in the Iraq, *S.typhimurium* by Saeed *et al*.,(16) and *S.ohio, S.newport* and *S.typhimurium* from cloacal swabs of chicken by AL-Iedani *et al*.,(23). In other countries, there were several studies supported this study for the isolation of most of these serotypes. One of studies that done in Spain by Capita *et al*.,(20) isolate *S.newport* and *S.typhimurium*, while in Iran by Dallal *et al*.,(14) isolate *S.typhimurium* with the *S. Thompson* (47%) was the most dominant serotypes. In China by Wang *et al*.,(19) isolate *S.typhimurium* with the *S. Indiana* (n=9) was the most common serotype.

The results of the study revealed that all *Salmonella* serotypes were (100%) resistant to at least one antibiotic and 16 (76.1%) have been resistant to three or more antibiotics (MDR) specially; *S. ohio*, *S. newport* and *S. typhimurium*. However, this finding agrees with Mansour *et al*.,(24) study in Iraq, which found that all *S.ohio* serotypes were MDR, and studies achieved in other countries; Italy (25), Spain (26), United State (27, 28, 29), United Kingdom (30), which were all reported that these three serotypes were MDR. All isolates were 100% susceptible to imipenem which were agree with study conducted in Iraq (24), in Iran (14, 15), which they found that all *Salmonella* serotypes were susceptible to imipenem. In the other hand, all serotypes were 100% resistant to cephalothin which was completely differ from finding of Mansour *et al*.(24) in Iraq, were found that no isolate resistant to cephalothin.

Higher levels of resistance found in this experiment may result in overuse of antibiotics in different chicken fields as for therapeutic or prophylactic purposes, which may create the pressure for the selection of antibiotic resistance in different bacterial pathogens (31, 5). Therefore, this study is recommended that the antibiotics should be administered at relevant doses for the required period after taking into account the antimicrobial susceptibility test results and require good strategies for controlling the organism during all stages of slaughtering.

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