Journal of Applied Veterinary Sciences, 8 (3): 22-29 (July, 2023)
ISSN: Online: 2090-3308, Print: 1687-4072
Journal homepage : https://javs.journals.ekb.eg

Isolation and Identification of Salmonella from Chickens Prepared for Slaughter in the State of Kuwait

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ABSTRACT

Salmonella enterica serovar Enteritidis throughout the world, is frequently linked to food-borne illness. Products made from poultry are important sources of transmission. Therefore, this study aimed to identify the incidence of *Salmonella* species in Kuwait's broiler flocks and determine which antibiotics are the most effective against the various *Salmonella* serotypes. A total of 2064 chicken samples (liver, intestine, and caecum) were collected from dead carcasses raised ten broiler flocks, between January and December 2017. The results revealed that *S. enterica* were found in the chicken older than 7 days old even though they did not have any signs of a clinical illness. It means that *S. enterica* can enter the human food supply through slaughterhouses and the contaminate carcasses. There was significant difference between the rate of *Salmonella* isolation and seasons, where the winter season had higher rate of *Salmonella* isolation compared to the other seasons. *Salmonella Enteritidis* and *S. typhimurium* were the most frequently isolated serotypes. Antimicrobials susceptibility testing showed that 88% to 60% of the isolates were sensitive to Amoxicillin Clavulanic acid. However, most of *Salmonella* isolates revealed high resistance to Cefotaxime, Ampicillin, Amoxicillin, Sulfamethoxazole trimethoprim followed by Gentamycin, Spiramycin, Doxycycline and Chloramphenicol. The study highlights the implementation of unique biosecurity and biocontrol strategies for Salmonella management that can prevent the negative effects of antibiotics and can make the environment and foods derived from animals safe.

Original Article:
DOI:https://dx.doi.org/10.21608/javs.2023.208149.1226

Received : 30 April, 2022.
Accepted :08 June, 2023.
Published in July, 2023.

J. Appl. Vet. Sci., 8(3): 22-29.

Keywords: Animal hygiene, Antimicrobials, Kuwait, Salmonellosis.

INTRODUCTION

Salmonella species are significant contributor to foodborne illnesses globally (**de Jong and Ekdahl, 2006**). Their yearly economic loss estimate is $3.7 billion (**USDA, 2016**). The main serovars linked to human salmonellosis are *Salmonella enterica* serovars Enteritidis and Typhimurium, which cause the majority of illnesses (**Dunkley *et al*., 2009**). In the United States, *S. enterica* represents the major cause of microbial foodborne diseases (**Scallan *et al*., 2011**). According to the Public Health Agency of Canada **(2006)** and Collard *et al*., **(2008)**, *S. enterica* serovar Enteritidis is mostly responsible for human salmonellosis outbreaks in the United States and Europe, and is associated with the ingestion of contaminated chicken products (**Braden, 2006; Much *et al*., 2009**). *Salmonella* species can cause self-limited, mild to moderate gastroenteritis, and in a rare number of the cases, severe disease may lead to death (**Voetsch *et al*., 2004**). *Salmonella* serotype prevalence varies between 6% and 30% in live birds (**Bjerrum *et al*., 2005**), while the incidence of *Salmonella enterica* in poultry and poultry products varies between 1% and 65.5% (**Agunos, 2007**).

For all ethnic groups, poultry and poultry products provide an affordable source of animal protein (**Nidaullah *et al*., 2017**). One of the most significant animal industries in Kuwait is the poultry sector, which provides around 50% of the chicken meat consumed in the State of Kuwait (**Ministry of planning. Statistical and Census Sector, 2020**). The most common poultry species worldwide is chicken. Additional, chicken meat is considered as Kuwait's main source of protein (**Ministry of planning. Statistical and Census Sector, 2020**). In Egypt, the prevalence of *Salmonella enteritidis* was (1.73%) and *Salmonella typhimurium* (0.43%) in imported ducklings (**Sorour *et al.*, 2023**). S*almonella* can spread horizontally and/or vertically in poultry. Examples of horizontal transmission include contamination from hatcheries, equipment, people, insects, rodents, feed, water, and litter (**Gast *et al.*, 2020**). According to Agunos (**Agunos, 2007**) and Crump and Mintz (**Crump and Mintz, 2010**), vertical transmission occurs when the bacteria is passed on directly from colonized breeding flocks (via ovaries) to the offspring.

Salmonella is thought to be mostly found in live chickens since it may live in their skin, feathers, and gastrointestinal tracts. *Salmonella* most frequently contaminates poultry meat through the processing of intestinal contents feces, infected processing tools, contaminated water, and processing employees' hands. In poultry slaughter plants, de-feathering, evisceration, chilling, and packaging processes can spread *Salmonella* throughout the entire plant (**Thames and Sukumaran, 2020**). Inadequate hygiene practices during chicken carcass cutting and preparation, poor cooking techniques, and temperature misuse, all contribute to human bacterial infection especially salmonellosis (**Ehuwa *et al*., 2021**). Typhoid fever, the most dangerous type of salmonellosis for humans, is still a big issue in the developing nations, largely because of the absence of sanitary and food safety standards (**Crump and Mintz, 2010; Akil and Ahmad, 2019**). Non-typhoid *Salmonella* is thought to be the cause of 93.8 million cases of mortality worldwide each year (**Antunes et al., 2016**). Eighty-three millions of these illnesses are thought to be foodborne (**Majowicz *et al*., 2010**).

Because of the high rate of cross-contamination during slaughter and processing, there are estimated risk levels for Salmonella outbreaks associated with these procedures in the broiler production process. These actions are thought to have a 12%, and a 33.5% chance of causing an outbreak, respectively (**Akil and Ahmad, 2019**). In addition, the main problem is that *Salmonella* infections in healthy chickens can occur without any outward signs of illness, which is why many farmers do not realize that their flocks are suffered from salmonellosis (**Beam *et al*., 2013**). Therefore, it is required to identify Salmonella infection in broiler flocks before slaughter (healthy chicken).

Furthermore, due to the significant costs associated with illness prevention, treatment, and control efforts, the rising frequency of salmonellosis has become a burden for most developing countries (**Lee *et al.*, 2015**). Additionally, due to the advent of novel serotypes and antibiotic resistance, researchers and society are now more aware of the wide variance in *Salmonella* serovars and the frequently shifting patterns in salmonellosis (**Antunes *et al*., 2016**). *Salmonella* has developed an antimicrobial resistance, mostly as a result of improper use of antibiotics in the poultry sector (**Sorour *et al.*, 2023**) and meat (**Oludairo *et al.*, 2022**). Therefore, this study was aimed to identify the incidence of Salmonella in Kuwait's breeding flocks; to determine the most prevalent serovars in the breeding flocks; to describe the seasonal variation in the occurrence of Salmonella, and to determine which antibiotics are the most effective against various *Salmonella* serotypes.

MATERIALS AND METHODS

Sampling

A total of 2064 chicken carcasses were collected from ten broiler flocks between January and December 2017 in Kuwait. After that, 4128 samples (2064 from liver; 2064 from intestine, and caecum) were collected from the same chicken. The age and number of birds were described in the table (1). All the samples were collected and transmitted in ice, sent to the Laboratories for Diagnosis Animal Diseases and Research immediately after death for isolation and identification of *Salmonella* species.

Table 1: The age and number of examined dead broilers

Age of dead broiler/day	Number of birds	Number of samples	
		Liver	Intestine and caecum
0-7	1584	1584	1584
7-14	262	262	262
14-30	218	218	218
Total Numbers	2064	2064	2064

Ethical approval

The ethical approval for this research design was not required.

Isolation and biochemical identification of *Salmonella* species

According to ISO 6579-2002, *Salmonella* from chicken and its relevant organs were isolated (ISO 6579, 2002). Chicken liver and intestinal samples were collected in an antiseptic environment as possible. Pre-enriching samples in buffered peptone water at a sample to broth ratio of 1:10 was followed by an 18-hour incubation period at 37°C. The pre-enriched broth was then utilized as an inoculum in Rappaport-Vasiliadis (RV) (EMD Chemicals Inc., Darmstadt, Germany), and incubated for 24 hours at 42°C. Rambach Agar (RA), Brilliance *Salmonella* Agar (BSA), and Xylose Lysine Deoxycholate Agar (XLD) (Difco, Sparks, NV, USA), which they utilized as selective agars, each received one loop of RV. The selective agar plates were then incubated for 24 hours at 37°C. The samples with red colonies (with or without black centers) on XLD, RA, and BSA were purified and placed through biochemical tests using the Analytical Profile Index (API-20E, BioMérieux, Inc., France).

Serological test

A slide agglutination test utilizing commercial antisera (SSI Diagnostica, Hillerod, Denmark) was used to serotype all biochemically verified Salmonella isolates based on somatic (O) and flagellar (H) antigens (**Popoff *et al*., 2004**).

Detection of multidrug resistant isolates

According to Clinical and Laboratory Standards Institute (2018), the sensitivity testing for bacterial isolates were carried out using the disc diffusion technique. To determine the susceptibility of the isolates, various Antimicrobials with varying concentrations (Amoxicillin (AML 10g), Ciprofloxacin (CIP 5g), Amoxicillin Clavulinic Acid (AMC 30g), Ampicillin (AMP 10g), Chloramphenicol (C 30g), Sulfamethoxazole Trimethoprim (SXT 25g), Gentamicin (GEN 10g), Spiramycin (SPI 100g), Cefotaxime (CTX 30µg), Doxycycline (DOX 30µg), Lincomycin (LIN10µg), and Clindamycin (CLI 2µg)) were used (Oxoid, Ireland).

Statistical analysis

The SAS Institute Inc., North Carolina, USA, JMP Pro16.1 software was used to perform descriptive and inferential statistics. The Chi-square test was used to examine the frequency of *Salmonella* species isolates and the seasonal patterns in order to determine the presence or abscent significant relationship. The results were significant at $P < 0.05$.

RESULTS

According to the morphological characterization, all *S. enterica* serovars isolated in the current study were motile, Gram-negative short rods or bacilli with red colonies, some of which had black centers on XLD, red colonies on RA, and purple colonies on BSA. All the isolates were negative for oxidase, urease, and indole, but positive for Lysine decarboxylase, methyl red, fermented mannitol, and glucose, and produced hydrogen sulfide (H_2S). Table (2) showed that *Salmonella* species was isolated from 2064 (16.2%) dead broilers at different ages, where the age of 0-7 days had the lowest number of isolates.

Table 2: Prevalence of *Salmonella* infection in according to the ages of chicken

Age /days	Total number of dead broilers (Liver and Intestine)	Positive samples	
		Number	%
0-7	1584	150^b	9.5
7-14	262	94^a	35.8
14-30	218	91^a	41.7
Total	2064	335	16.23

Within the same column, frequencies with various letters are significantly different ($P < 0.05$).

Our findings showed that there was significant difference between the rate of Salmonella isolation and seasons and winter season had a higher rate of Salmonella isolation compared to the other seasons as summarized in (Table 3).

Table 3: The rate of Salmonella isolation during the different seasons

Season	No. of dead broilers/ season	Positive *Salmonella* species	
	Liver and Intestine	Number	Percentage
Winter	591	138[a]	23.3
Spring	510	80[b]	15.7
Summer	555	72[bc]	13
Autumn	408	45[c]	11

Within the same column, frequencies with various letters are significantly different ($P < 0.05$).

Various serotypes isolated from different ages of dead broilers were recorded in Table (4), where *S. Enteritidis* showed the highest rate of isolation (40.9%), followed by *S. Typhimurium* (29.6%), *S. Thompson* (22.1%), and *S. Munchen* (7.4%).

Table 4: The serotypes of *salmonella* isolated from dead broiler's liver and intestine samples

Age /day	*Salmonella* species isolates	*S. Typhimurium* No. %	*S. Enteritidis* No. %	*S. Thomson* No. %	*S. Munchen* No. %
0-7	150	50 (33.3%)	50 (33.3%)	30 (20%)	20 (13.3%)
7-14	94	15 (15.9%)	64 (68.1%)	10 (10.6%)	5 (5.4%)
14-30	91	34 (37.4%)	23 (25.3%)	34 (37.3%)	0 (0%)
Total	335	99 (29.6 %)	137 (40.9%)	74 (22.1%)	25 (7.4%)

In additionally, this study revealed that 80% of *S. Enteritidis* isolates were sensitive to Amoxicillin Clavulanic acid (AMC). On the other hand, most of *S. Enteritidis* were resistance to Cefotaxime (CTX) 100%, Ampicillin (AMP) 94%, and Amoxicillin (AML) 87.5% (Table 5).

Table 5: Antimicrobial sensitivity testing of the isolated Salmonella Enteritidis

Antimicrobials	Conc. (µg)	Resistant		Intermediate		Sensitive	
		No.	%	No.	%	No.	%
Ciprofloxacin	5	20	40	7	14	23	46
Chloramphenicol	30	7	14	16	32	27	54
Doxycycline	30	37	74	5	10	8	16
Gentamycin	10	44	88	2	4	4	8
Spiramycin	100	28	56	6	12	16	32
Sulfamethoxazole trimethoprim	25	50	100	0	0	0	0

Furthermore, the findings revealed that 60% of *S. Typhimurium* isolates were sensitive to Amoxicillin Clavulanic acid (AMC). On the other hand, most of *S. Typhimurium* were resistance to Cefotaxime (CTX) 100%, Ampicillin (AMP) 94%, and Amoxicillin (AML) 96% (Table 6).

Table 6: Antibiogram of the isolated *S. Typhimurium*

Antibacterial agent	Conc. (µg)	Resistant		Intermediate		Sensitive	
		No.	%	No.	%	No.	%
Ciprofloxacin	5	10	0	4	50	36	50
Chloramphenicol	30	15	30	5	10	30	60
Doxycycline	30	40	80	0	0	10	20
Gentamycin	10	29	58	2	4	21	42
Spiramycin	100	30	60	9	18	11	22
Sulfamethoxazole trimethoprim	25	49	98	1	2	0	0

Moreover, our results revealed that 88% of *S. Thomson* isolates were sensitive to Amoxicillin Clavulanic acid which was the best of choice. But, most of *S. Thomson* were resistance to Cefotaxime (CTX) 100%, and both Ampicillin (AMP), and Amoxicillin (AML) 98% (Table 7).

Table 7: Antibiogram of the isolated *S. Thomson*

Antibacterial agent	Conc. (μg)	Resistant		Intermediate		Sensitive	
		No.	%	No.	%	No.	%
Ciprofloxacin	5	0	0	4	50	4	50
Chloramphenicol	30	25	50	0	0	25	50
Doxycycline	30	40	80	4	8	6	12
Gentamycin	10	31	62	5	10	14	28
Spiramycin	100	34	68	2	4	14	28
Sulfamethoxazole trimethoprim	25	48	96	2	6	0	0

Lastly, the results revealed that 60% of *S. Munchen* isolates were also sensitive to Amoxicillin Clavulanic acid which was the best of choice. The results also pointed to the high resistance of *S. Munchen* to Ampicillin, Cefotaxime and Sulfamethoxazole Trimethoprim (100%) followed by Gentamycin, Amoxicillin, Spiramycin, Doxycycline and Chloramphenicol (96%, 92%, 84%,80%, and 52% respectively).

DISCUSSION

One of the most common foodborne infections in humans is salmonellosis. The constant threat to human health arises from the ongoing presence of *Salmonella* spp. in animals raised for food and the increasing resistance to antimicrobial drugs. The chicken sector suffers significant financial losses due to Salmonella. Salmonella infections in poultry flocks cause considerable direct losses for poultry producers. Young chicken may experience growth retardation, or even death as a result of infections they acquire either horizontally in the hatchery or vertically from their parents. For chicken breeders, preventing the spread of Salmonella to the offspring or to the people can be costly (**Sohail *et al*., 2021**). In the current study, the broilers that prepared for slaughter was examined for the presence of Salmonella.

Although chickens may be infected with *Salmonella* at any age (**Friedman *et al*., 2003**), the present results revealed that 14-30 days old broilers (41.74%) were more susceptible to Salmonellosis. This variation in susceptibility may be due to the presence or absence of the chicken innate immunity which is the first barrier against any infection (**Cheema *et al*., 2003**).

Regarding the seasonal variation, out results were in agreement with the study in Japan, by **Ishihara *et al.,*** (**2020**) who found that *Salmonella* spp. was higher during the winter and spring than the other seasons. However, our findings disagreed with **Zdragas *et al.,* (2012)** who reported that there was seasonal variation of *Salmonella* isolation. Its peak was recorded in summer than winter. Additionally, depending on the age of the chicken, the air temperature in broiler houses must be strictly regulated. In other words, it's possible that Salmonellosis spread outside or inside the broiler houses is not the cause of the higher risk of Salmonella isolation from chicken in the winter and spring. On the other hand, the management practices, such those impacting ventilator or the time frame for opening window̓s coverings in chicken houses, should be adjusted according to the season (**Ishihara *et al.,* 2020**). The types and numbers of Salmonella carriers into broiler flocks, such as hygienic insects or wild animals, would fluctuate based on the temperature of the air outside the broiler houses.

The broilers aged 7 - 14 days had the highest isolation rate of *S. Enteritidis* (68.1%), whereas no *S. Munchen* could be isolated from the broilers aged 14 - 30. However, the prevalence of *S.* enteritidis was estimated to be 6.3% in Uruguay as mentioned by Betancor *et al*., (**2010**). Previous investigations have shown that raw chicken meat can contain *Salmonella* enterica as documented by Amin and Abd El-Rahman, **(2015);** Soguilon-Del Rosario and Rivera, (**2015**). *Salmonella Enteritidis* and *S. Typhimurium* are introduced independently from other *Salmonella* sero-types for two reasons: first, because the epidemiology of these bacteria differs from other *Salmonella* spp., second, because they are the most frequently sero-types linked to this human disease in the majority of countries as summarized by Bangtrakulnonth *et al*., **(2004**).

Antimicrobials susceptibility testing showed that 88% to 60% of the isolates were sensitive to Amoxicillin Clavulanic acid drug. These results disagree with Rodrigues, *et al.*, **(2017)** who found that 66.7% of the isolated Salmonella in West-Center regions of Brazil was resistance to Amoxicillin Clavulanic acid. The use of Beta-lactams in animal feed as a growth performance additive, in addition, to the miss use of antibiotics in poultry farms may lead to the presence of Beta-lactamas resistant Salmonella. In this study, *Salmonella* isolates showed resistance to more than 3 classes of antibiotics, and these results agreed with Elkenany *et al*., (**2019**) and Zhang *et al*., (**2018**).

CONCLUSION

The findings of the current study revealed that *S. enterica* infection causes a persistent enteric infection in chickens older than 7 days without manifesting clinical signs and serves as a source of contamination for carcasses at the point of slaughter and entry into the human food supply. The seasons had noticeable impact on the rate of *Salmonella* isolation. The two most prevalent and isolated serotypes from the broilers were *S. Enteritidis* and *S. Typhimurium*. The study also found a higher incidence of *Salmonella* that is resistant to antibiotics. It requires immediate action to reduce the use of antibiotics and to take biosecurity precautions that would help to prevent the spread of Antimicrobials resistant *Salmonella*. Due to the spread of these strains in broiler farms, specific biocontrol measures were necessary to control Salmonellosis. These actions can prevent the negative effects of antibiotics and guarantee the environmental and food safety generated from animals.

ACKNOWLEDGEMENTS

We wish to express our gratitude, and value their assistance of all the herd owners who took part in the study.

Conflicts of interest

The authors of the present manuscript states that neither the writing nor the data analysis had any conflicts of interest.

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How to cite this article:
Ahmad Dh. Alajmi, Omar H. Sheet, Omar A. Al-Mahmood, Islam M. Saadeldin, and Raad A. Alsanjary, 2023. Isolation and Identification of Salmonella from Chickens Prepared for Slaughter in the State of Kuwait**.** Journal of Applied Veterinary Sciences, 8 (3): 22-29.
DOI:https://dx.doi.org/10.21608/javs.2023.208149.1226