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Detection some Antimicrobial Resistance Genes in *Salmonella sp.* Isolated from Chicken Meat

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***Abstract*— Salmonellosis is a disease condition caused by a large group of bacteria of the genus *Salmonella* that can affect human being throughout the world. Fresh and processed poultry have been frequently implicated in cases of human salmonellosis. Furthermore, increased consumption of meat and poultry has increased the potential for exposure to *Salmonella* enterica. *Salmonella* is one of the leading causes of food-borne diseases. The present study was designed in order to estimate the prevalence of *Salmonella spp.* in chicken meat in Nasiriyah city (Iraq) and detection of some antimicrobial resistance gene.**

The period of specimens collection extended from March 2019 to July 2019. One hundred and twenty five (frozen chiken chest meat) collected from the markets of Nasiriyah city. This study showed that out of (125) studied specimens (16) specimens were *Salmonella* positive (12.8 %). *Salmonella* were isolated and identified by using bacterial culturing on buffered peptone water, tetrathionate broth, XLD, S.S.Agar, Nuterinte broth and Nutrient agar and confirming tests by API 20E system as well as molecular diagnosis by using and *invA* genes for *Salmonella*, all results of these diagnosis methods referred to all isolates belong to *Salmonella spp. Salmonella* isolates from chicken have been tested for their antibiotic resistance against (10) different antibiotics using the Kirby-Bauer Dissemination Method. All chicken *Salmonella* isolates are sensitive to Gentamycin ,while were (87.5%) isolates are resistances to Ciprofloxacin and Nalidixic acid , (56.25%) isolates are resistances to Cefotaxim , (43.75%) isolates are resistances to Norfloxacin and Amoxicillin- clavulanic acid , (18.75%) isolates are resistances to Cefixim , (6.25%) isolates are resistances to Amikacin and Azithromycin. The percentage of multidrug-resistant (62.5%) of chicken *Salmonella* isolates had multi drug resistance. The isolates were tested for the presence of antibiotic resistance genes using traditional polymerase chain reaction (PCR) to identify and antibiotic resistance genes in *Salmonella* The genes: (*gyrA*, *parC*, *qnrA*, *qnrS* and *qnrB)*. This study found in isolates of chicken *Salmonella* isolates as follows: *gyrA* gene expose in 15/16 isolates (93.75%), *parC* gene expose in 15/16 isolates (93.75%), *qnrB* gene expose in 1/16 isolates (6.25%).

***Keywords*— *Salmonella spp.*, Antimicrobial resistance, chicken meat.**

I. Introduction

Salmonella is a Gram negative bacterium belonging to The family Enterobacteriaceae, and known as "enteric" bacteria. *Salmonella* are found in the intestinal tract of animals and humans (Jaran, 2015). *Salmonella* species are known as zoonotic pathogens which cause diseases in both humans and animals. They are the etiological agents of severe clinical manifestations in humans(Hossain *et al.*, 2019). These Microorganisms may cause diseases in either of the two ways intoxication or replication of microorganisms. Intoxication involves pathologic changes in the host caused by toxin formed before ingestion of the microorganism while food born infection, on the other hand, results from replication of microorganisms after it has been ingested (Neges and Abebe, 2018). Poultry, especially the meat (chicken meat) which is known to be avery good source of protein with low fat content having little the best source of animal protein for the low income populations since it is inexpensive and within reach. Because of these advantages، large scale consumption of poultry meat is greater than that of other meats. the major source of *Salmonella* infections in human is via the ingestion of chiken meats. Thus، ensuring the microbial safety of chiken meat products is highly important so as to assure a healthy production and consumption of the meat.

Furthermore, contamination of the chiken meat arises during and after slaughtering either from the animal microbiota, the slaughter houseenvironment and the equipment used during the processing processes, and some of these bacterial contaminants can grow or survive during food processing and storage (Callejón *et al.*, 2015; Wemedo, Douglas and Nima, 2019). *Salmonella* cause about 80% of infections in human globally Infections by *Salmonella* species in human caused by uncooked of meat poultry (Sadeq, Esmaeel and Neama, 2017). Many *Salmonella* serovars exist. More than 2,600 serovars are classified based on the reactivity of antisera to the somatic O , flagellar H antigens and and capsular Vi antigens (Velege, Cloeckaert and Barrow, 2005; Zhao *et al.*, 2017). The genus *Salmonella* consists of only two species, *Salmonella enterica* and *Salmonella bongori*. *S. enterica* is divided into six subspecies: *S. enterica subsp. enterica*, *S. enterica subsp. salamae*, *S. enterica subsp. arizonae*, *S. enterica subsp. diarizonae*, *S. enterica subsp. houtenae, and S. enterica subsp. Indica* (Issenhuth-Jeanjean *et al.*, 2014) .

The increasing prevalence of multidrug resistance among *Salmonella* and resistance to clinically important antimicrobial agents has also been an emerging problem in countries (Lamas *et al.*, 2015). Horizontal Gene Transfer (HGT) is the transfer of genetic material to bacteria from the same generation and a successful HGT relies on the introduction of DNA into a recipient cell's cytoplasm and heritability of the transferred sequences in the recipient microorganism. HGT consists of conjugation, transduction, and transformation with mobile genetic elements, like conjugative elements، insertion sequences، transposons، miniature inverted-repeat transposable elements, as the major contributor in these genetic transformations, which are able to share genetic information between bacteria (Shariati *et al.*, 2018). MDR *Salmonella* can be transferred from chiken to humans through the food chain or by physical contact (Firoozeh *et al.*, 2012). Aim of study was designed in order to estimate the prevalence of *Salmonella spp.* in chicken meat and detection of some antimicrobial resistance gene.

II. Materials and Methods

A. Collection samples

One hundred and twenty five samples of domestic and imported chicken meat were collected in The markets of Nasiriyah city During the duration of March 2019 until July 2019.

B. Isolation of Salmonella bacteria from Chiken meat:

Weighed 25 g of each sample and was added to 225 ml From Nuterinte broth then Incubated at 37 ° C for 18-24 hours the Transfer 1 ml to the medium Tetrathionate broth TTB After the growth of the bacteria were planted on the media Solid Deoxychoglate(XLD) incubated at 37 ° C for 18-24 hours then took the perfect colony to hold biochemical tests (AL-mossawei, Kadhim and Hadi, 2015).

C. Identification of the Isolates by API 20 E System.

This test was performed according to (BioMerieux, France); it is used clinically for the rapid identification of *Enterobacteriaceae*. This test consists of 25 plastic strips and each strip contains 20 small tubes with upper orifice (Cupule) and lower orifice (tube) containing dried material and representing a biochemical test, color changes occurring in the tubes either during incubation or after incubation.

D. Molecular diagnosis invA gene by PCR

The PCR for invA gene-specific oligonucleotide primers for the invA gene is listed in Table 1.

Table 1: Sequences of primers used for *invA* gene amplification

Gene name	Primer Sequences (5'-3')		program	cycle	Product Size(bp)	Reference
InvA	F	CTGGCGGTG GGTTTT- - GTTGTCTTC TCTATT	95^{O}C, 5min. 94^{O}C, 40sec. 66.5^{O}C, 60sec. 72^{O}C, 90 sec. 72^{O}C, 10 min.	35	1070	(Galan and Curtiss, 1989)
	R	AGTTTCTC CCCCTCT- - TCATGCGT TAC				

E. Antimicrobial Susceptibility Test

The antimicrobial susceptibility test was conducted by disc diffusion technique . Many kinds of antibiotic disks have been chosen to detect antimicrobial susceptibility to *Salmonella spp*. isolates. Table(2) shows the antibiotics disks used in this study.

Table (2): Antibiotics Disks used in the present study.

No.	Antibiotic	Symbol	Concentration µg.
1	Amikacin	AK	30
2	Amoxicillin- clavulanic acid	AMC	30
3	Azithromycin	AZM	15
4	Cefixim	CFM	5
5	Cefotaxim	CTX	30
6	Ciprofloxacin	CIP	5
7	Gentamicin	CN	10
8	Nalidixic acid	NA	30
9	Norfloxacin	NOR	10
10	Tetracycline	T	30

F. Polymerase Chain Reaction Assay

Genomic DNA was extracted from *Salmonella* isolates using the Geneaid Genomic DNA Purification Kit (UK) and performed as directed by the business. Genomic DNA extracted is checked using a Nano-drop spectrophotometer which measures the concentration of DNA (ng/µl) and checks the purity of DNA by reading the absorbance at (260/280 nm).

G. Detection of , gyrA , parC, qnrS, qnrA and qnrB genes by PCR assay

Primers used in this study was purchased from alphadna company (canada) in lyophilized form. Sequences of primers used for gene amplification show in Table 2.

After preparing the reaction volume in PCR tube the mixture was spin down and then PCR tube placed in the PCR thermo cycler and the amplification reactions was started according to the program described in the Table3.

Table 3: Sequences of primers used for gene amplification

Gene name	Primer Sequences (5'-3')		Program	cycle	Product Size(bp)	Reference
gyrA	F	TGGGCAATGACTGGAA--CA	95°C, 3 min. 95°C, 45 sec. 55°C, 45 sec. 72°C, 50 sec.	30	431	(J. Wang *et al.*, 2017)
	R	GGTTGTGCGGCGGGATA	72°C , 10min.			
parC	F	ATGAGCGATATGGCA--GAGCG	95°C, 3 min 95°C, 45 sec 61.5°C, 45 sec	30	413	(Giraud *et al.*, 1999)
	R	TGACCGAGTTCGCTT--AACAG	72°C, 50 sec 72°C , 10 min			
qnrB	F	GATCGTGAAAGCCAGA-AAGG	95°C, 3 min 95°C, 45 sec	30	469	(Cui *et al.*, 2015)
	R	ACGATGCCTGGTAGT--TTCC	61°C,45 sec 72°C, 50 sec 72°C , 10min			
qnrA	F	ATTTCTCACGCCAGGAT--TTG	95°C, 3 min 95°C, 45 sec 59°C,45 sec 72°C, 50 sec 72°C , 10min	30	516	(Cui *et al.*, 2015)
	R	GATCGGCAAAGGTTAGG--TCA				
qnrS	F	ACGACATTCGTCAACTG--CAA	95°C, 3 min 95°C, 45 sec 59°C,45 sec 72°C, 50 sec 72°C , 10min	30	417	(Cui *et al.*, 2015)

The PCR products of was analyzed by agarose gel electrophoresis, The agarose gel was prepared by dissolving 0.3 gm of agarose powder in 25 ml of (1x) TBE buffer (PH 8.0), the solution was heated on microwave, until all crystals were dissolved in agarose , after that, cooling to 60°C, (0.5 µg ⁄ ml) ethidium bromide was added mixed with it. Then the comb was fixed in the right position and the gel was poured in the tray and left until solidifying. Then the comb removed carefully.

The gel was transferred into electrophoresis machine which contained the TBE buffer that used in preparation of agarose gel. PCR product of 5µl was pipetted into each well, and 2.5 µl of (100 bp ladder) added in the first well to use as a molecular marker to estimate the size of the PCR products. Electric current was set up at 50 Volt 85 min1hour. Finally PCR products were visualized by using UV Transilluminator.

III. RESULTS

A. *Isolation and Identification of Salmonella spp.*

A total of 125 of frozen chiken meat have been collected and tested from March 2019 to July 2019. revealed that *Salmonella spp* in Fig.1. Occurred in 12.8 % for food.

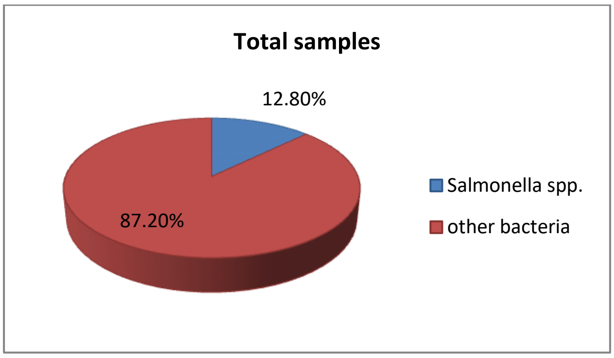

Figure (1) :the percentage of *Salmonella* isolates.

B. *Morphological properties*

The results showed the different morphology characteristics of all *Salmonella spp* which grow on different media as in Table 4.

Table 4: Culture characteristics of *Salmonella spp.*

Culture Media	Morphology of colonies
Xylose-Lysine Deoxycholate agar(XLD)	Small, smooth, rounded, red in color with black center
Salmonella-Shigella agar (S.S.Agar)	Small, smooth, rounded, pale with black center
Nutrient agar(NA)	Small, smooth, rounded and pale

Figure (2) : *Salmonella* Growth on Different Media: XLD Agar

C. *Identification by using API 20 E system*

All the isolates have been tested by API 20E system for confirmation of the identification .The results showed that of 32 isolates *Salmonella spp.* as shown in Table 5.

Figure (3) : Calculate the numerical profile in Api-20E system

Table (5): API 20E test of *Salmonella spp.*

Biochemical Test	results	Biochemical Test	results
ONPG (β-galactosidase)	-	GEL (Gelatinase)	+
ADH (Arginine dihydrolase)	+	GLU (Glucose)	+
LDC (Lysine decarboxylase)	+	MAN (Mannitol)	+
ODC (ornithine decarboxylase)	+	INO (Inositol)	+
CIT (Citrate utilization)	+	SOR (Sorbitol)	+
H2S (H2S production)	+	RHA (Rhaminose)	+
URE (Urease test)	-	SAC (Sucrose)	-
TDA (Tryptophan deaminase)	-	MEL (Melibiose)	+
IND (Indole test)	-	AMY (Amygdaline)	-
VP (Acrtoin production)	-	ARA (Arabinose)	+
Code	**6706752**		
Diagnosis	***Salmonella spp.***		

D. *Multi-drug Resistance Pattern of Salmonella Isolates from Chicken meat Samples.*

This study shows that 62.5% of *Salmonella spp.* isolated from chicken meat samples are considered as multi-drug resistant, two of the isolates (12.5%) were resistant to four antibiotics, four of the isolates(25%) were resistant to five antibiotics, three of the isolates (18.75%) were resistant to six antibiotics and one of the isolates(6.25%) were resistant to eight antibiotics As shown in Table (6) and Figure (2).

Table (6) : MDR *Salmonella spp* in chicken isolates.

No .of antibiotic	*Salmonella spp* (n=16) in chiken isolates			
	Multiple Resistance patterns	**Isolates**		**Total (%)**
		No.	**(%)**	
Four	CIP+NOR+NA+T	1	6.25	2(12.5%)
	CIP+NOR+CTX+NA	1	6.25	
Five	CIP+NOR+CTX+NA+T	1	6.25	4(25%)
	CIP+NOR+CTX+NA+T	1	6.25	
	CIP+AMC+CTX+NA+T	1	6.25	
	CIP+NOR+CTX+NA+T	1	6.25	
Six	CIP+CFM+AMC+CTX+NA+T	1	6.25	3(18.75%)
	CIP+CFM+AMC+CTX+NA+T	1	6.25	
	CIP+CFM+AMC+CTX+NA+T	1	6.25	
Eight	CIP+AK+AMC+NOR+AZM+CTX+NA+T	1	6.25	1(6.25%)

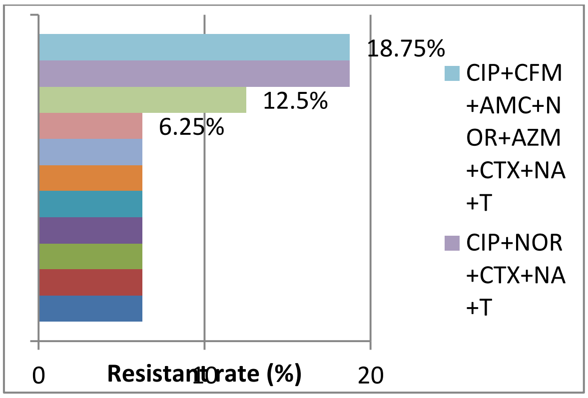

Figure (4): resistant rate in chicken *Salmonella* isolates.

E. *Molecular diagnostics*

Simplex PCR is used to detect the presence of antibiotic resistance genes were *gyrA* , *parC* and *qnrB* genes in *Salmonella* isolated from frozen chicken chest meat .The sizes used in this analysis of these genes: *gyrA* gene (431bp) , *parC* gene (413bp) and *qnrB* gene (469bp). Each gene is defined as a single band in the corresponding DNA ladder region.

1) *Molecular Diagnosis of Salmonella spp.*

All of 16 *Salmonella spp* identified by conventional biochemical tests and API 20E were subject to DNA extraction and PCR tests for the presence of *invA* genes in succession . isolates were positive results in 16(100%) of *invA* genes as shown in Fig. (5).

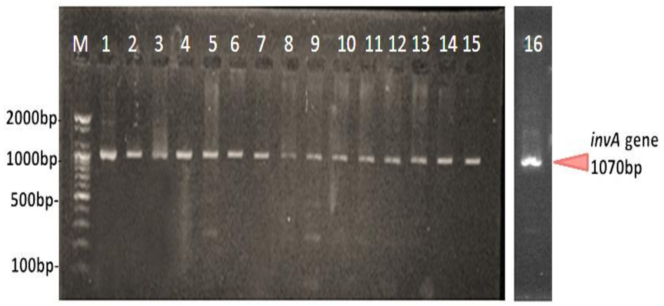

Figure (5): Gel electrophoresis of amplified *invA* gene, the product size 1070 bp of *Salmonella spp.* using conventional PCR. Agarose 1.2%, and TBE (1X) at (50V for 85 mins. Lane (M): DNA ladder(100-2000bp), Lanes:(1 – 16) positive samples.

F. Molecular Detection of Antibiotic Resistance Genes in Salmonella spp. Isolated from Chicken Meat.

1) gyrA gene

All isolates of *Salmonella* from chicken meat it was found that *gyrA* gene expose in 15/16 isolates (93.75%) as shown in Fig. (6)

Figure (6): Agarose Gel Electrophoresis image of *gyrA* gene, the product size 431 bp of chicken *Salmonella spp.* using conventional PCR. Agarose 1.2%, and TBE (1X) at (50V for 85 mins. Lane (M): DNA ladder(100-3000bp), Lanes:(1–6;8-16) positive samples while (7) negative samples .

2) parC gene

All isolates of *Salmonella* from chicken meat it was found that *parC* gene expose in 15/16 isolates (93.75%) as shown in fig.(7).

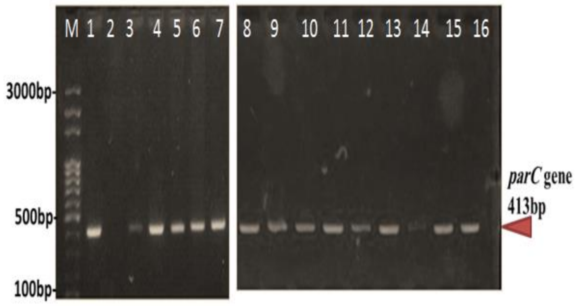

Figure (7): Agarose Gel Electrophoresis image of *parC* gene, the product size 413bp of chicken *Salmonella spp.* using conventional PCR. Agarose 1.2%, and TBE (1X) at (50V for 85 mins. Lane (M): DNA ladder (100-3000bp), Lanes:(1,3-16) positive samples while (2) negative samples.

3) qnrB gene

All isolates of *Salmonella* from chicken meat it was found that *qnrB* gene expose in 1/16 isolates (6.25%) as showin fig. (8).

Figure (8): Agarose Gel Electrophoresis image of *qnrB* gene, the product size 469bp of chicken *Salmonella spp.* using conventional PCR. Agarose 1.2%, and TBE (1X) at (50V for 85 mins. Lane (M): DNA ladder(100-3000bp), only (3) positive samples.

G. qnrA and qnrS gene

The results of this study showed that all isolates of chicken *Salmonella* do not contain *qnrA* and *qnrS* gene gene.

H. The prevalence of (qnrA ,qnrS , gyrA , parC & qnrB) genes and phenotype of antibiotic resistance Chicken meat Salmonella isolates.

Figure 9 shows the percentages of genes that appeared in the deportation and the tables (7) illustrate the spread of genes and their relationship to antibiotics for *Salmonella* isolates from chicken meat, as shown by the results of this study.

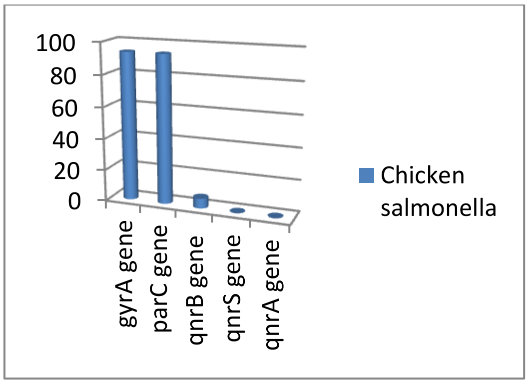

Figure (9): Percentages of genes in *Salmonella* isolates isolated from chicken meat.

Table (7): result of antibiotic resistance genes detection of chicken *Salmonella***.**

Isolate type	antibiotic resistance genes					MDR
	qnrS	*qnrA*	*gyrA*	*parC*	*qnrB*	
Chicken sample	–	–	+	+	–	CIP+NOR+NA+T
Chicken sample	–	–	+	–	–	Non
Chicken sample	–	–	+	+	+	CIP+AK+AMC+NOR+AZM+CTX+NA+T
Chicken sample	–	–	+	+	–	CIP+NOR+CTX+NA+T
Chicken sample	–	–	+	+	–	Non
Chicken sample	–	–	+	+	–	Non
Chicken sample	–	–	–	+	–	Non
Chicken sample	–	–	+	+	–	CIP+NOR+CTX+NA+T
Chicken sample	–	–	+	+	–	Non
Chicken sample	–	–	+	+	–	CIP+NOR+CTX+NA+T
Chicken sample	–	–	+	+	–	CIP+AMC+CTX+NA+T
Chicken sample	–	–	+	+	–	CIP+NOR+CTX+NA
Chicken sample	–	–	+	+	–	CIP+NOR+CTX+NA+T
Chicken sample	–	–	+	+	–	CIP+CFM+AMC+CTX+NA+T
Chicken sample	–	–	+	+	–	CIP+CFM+AMC+CTX+NA+T
Chicken sample	–	–	+	+	–	CIP+CFM+AMC+CTX+NA+T

IV. Discussion

Salmonellosis is a major cause of human bacterial gastroenteritis thatrepresents a growing public health concern in both developing and developed countries Alali *et al.*, (2012); Almashhadany, (2019) . *Salmonella spp*. is among the most important food borne pathogens in the world. Poultry and poultry products are usually causing human salmonellosis outbreaks. Chicken products are widely acknowledged to be a significant reservoir for *Salmonella*. They have frequently been incriminated as a source of *Salmonella* contamination and consequently thought to be major sources of the pathogen in humans AL-Jobori, Hasan and Nader,(2016).

The results of bacterial cultures obtained in this study showed that the total range of *Salmonella* isolates from frozen chicken meat (local and imported) were16/125 (12.8%) These results were agree to the findings in Egypt Orady *et al.*, (2017) (12.8%) and is also compatible with a study conducted in Turkey,Özbey and Ertas, (2006) (12%) the presence of *Salmonella* spp. Isolation from chicken samples.

These results were agree to the findings in India Naik *et al.*,(2015) (12.5%) and were almost similar to the findings Dahal, Ellerbroek and Poosaran,(2007) (13%) the presence of *Salmonella spp.* isolation from chicken samples in India.

The result of the current study is not consistent with a study Qader and AlKhafaji, (2019) in Baghdad that found the percentage of *Salmonella* bacteria isolated from chicken meat (8%).

While a study AL-Jobori, Hasan and Nader,(2016) found a high percentage (38%) of *Salmonella* isolated from breast of chicken.

The difference in results between the current and previous studies and the prevalence of salmonella at different rates is related to several reasons:

1.showed that *Salmonella spp.* was widespread among the chicken may be due that the defeathering process may spread microorganisms between carcasses or from the defeathering equipment contributing to an increase in the numbers of psychrotrophs and aerobe mesophiles on the carcasses. The evisceration process provides an opportunity for cross contamination from human, equipments and worker's hands Jackson, Peres-Neto and Olden,(2001)

2.As well as poor hygiene conditions, regarding the temperature of storage, the equipment and the employees' personal hygiene. The cutting tables were seldom washed or disinfected before use. These benches could therefore be reservoirs from which *Salmonella* could spread to. other equipment through flies or direct contact Stevens *et al.*, (2006)

3.Differences in the prevalence rate of *Salmonella* isolates with the previous study may be attributed by the multiple factors, such as geographic and seasonal variation, variations in sampling animal size، and sample procedure management practices, hygienic conditions during production and processing of meat and meat products or due to differences in the sensitivity and specificity of different isolation methods used Bhoomika *et al.*, (2019).

The present study *Salmonella* isolates have shown that all of the isolates (100%) were sensitive to Gentamycin The result of this study is compatible with a study Kaya *et al.*(2017), It was found that all of the isolates (100%) were sensitive to Gentamycin

The present study , a very high rate (87.5%) of nalidixic acid resistance was observed in *Salmonella* isolates, which is similar to the rate (88.95%) found in , China Cui *et al.*, (2016). On other hand it is similar to the rate (89.28%) found in Turkey Siriken *et al.*, (2015), While in the study Orady *et al.*, (2017), in Egypt found that the percentage (93.7%) of higher than the current study isolated *Salmonella* resistance to nalidixic acid.

While in the study Abd-elghany *et al.*(2015), Mansoura, Egypt showed the percentage (98.8%) higher than the current study that *Salmonella* isolates are of nalidixic acid resistance.

current study, a very high rate (87.5%) of ciprofloxacin resistance was observed in *Salmonella* isolates, The current study is therefore incompatible with one study

Hameed, Abd and Abbas, (2014)in Al-Najaff and Al-Hilla Provinces found that the percentage (73.3%) of *Salmonella* isolates from chicken meat resistant to ciprorofloxacin is lower than my current study.

Current study, high rate (62.5%) of tetracycline resistance was observed in Salmonella isolates, The current study is lower than percentage than study Ramatla *et al.*, (2019)in North West, South Africa (68%) resistant of all isolate *Salmonella* isolated from chicken resistance to tetracycline.

Also, there is another study by Parveen *et al.*, (2007)showed the percentage of *Salmonella* isolates resistant to tetracycline (73.4%) higher than the percentage in current study. The results reported in this study higher than those found by Santos *et al.*(2000)in Brazil (6.25%), and also by Antunes *et al.*(2003) in Porto, Portugal (36%).

This study, showed high rate (43.75%) of norfloxacine resistance was observed in *Salmonella* isolates was higher than study Cardoso *et al.*(2006) in Brazil (0%) resistant of all isolate *Salmonella* isolated from chicken resistance to norfloxacin.

The results in this study, indicated high rate (43.75%) of norfloxacine resistance was observed in *Salmonella* isolates was higher than study Abd-elghany *et al.*(2015) in Egypt (30.1%) *Salmonella* isolated from chicken meat resistant to norfloxacin and approach to the percentage in the study Wajid *et al.*,(2019)in Pakistan (47%) that isolated *Salmonella* from Poultry Farms.

The results of the current study showed the percentage of *Salmonella* isolates resistant to amikacine (6.25%) to be similar to the percentage in the study Yu *et al.*(2014) in Henan, China (3.2%) of all isolate *Salmonella* isolated from chicken resistance to amikacine and lower than percentage Y. Wang *et al.*, (2017) of *Salmonella* isolates from chicken meat (48.2%)in china resistant to amikacine.

the percentage of *Salmonella* isolates in this study resistant to cefixime (18.75%) larger than study Bhoomika *et al.*(2019) showed *Salmonella* (7.40%) that resistance to cefixime from chicken meat in Chhattisgarh. This study found percentage of *Salmonella* isolates resistant to cefixime lower than study in india Naik *et al.*,(2015) isolation *Salmonella* (81.25%) resistance to cefixime from chicken meat.

The results for detection the percentage of *Salmonella* isolates resistant to cefotaxime (56.25%) are larger than study Y. Wang *et al.*, (2017) in china found (44.7%) of *Salmonella* isolates from chicken resistane to cefotaxime and larger than study Yildirim *et al.*, (2011)in Anatolia found (2.9%) of *Salmonella* isolates from chicken resistane to cefotaxime.

Illustrated of this study found the percentage of *Salmonella* isolates resistant to azethromycine (6.25%) are lower than study Teixeira, Lima and Oliveira, (2016) in Northeast Brazil (48.8%) of *Salmonella* isolates from chicken resistane to azethromycine , lower than percentage of study Fatema *et al.*, (2014) in Bangladesh (100%) of *Salmonella* isolates from chicken resistane to azethromycine and lower than study Ahmed *et al.*, (2018)in Koya city (83.3) of *Salmonella* isolates from chicken feces resistane to azethromycine.

The high occurrence of the antibiotic-resistant *Salmonella* strains from indigenous chickens could be due to a pick-up of antibiotic resistance and virulence genes determinants from the environment or through interaction hosts such as rodents and livestock whom they share feeding and drinking troughs.

Chicken *Salmonella* 16 isolates (62.5%) showed multidrug resistance phenotypes to at least four classes of antimicrobials. The percentage of multidrug-resistant *Salmonella* strains is differ from that reported in Italy (2.3%) Nastasi, Caterina and Cannova, (2000), Iran (23.5%) Soltan-dallal *et al.*, (2009) and that found (100%) by Yildirim *et al.*,(2011), in Turkey.

This study, PCR assay was carried out for the detection of the *invA* gene from 16 isolates from chicken meat , the results of the current study revealed that the gene was present in all of the isolates(100%) which was in agreement with the study Byomi *et al.*, (2019) and Deguenon *et al.*, (2019) and was in agreement with the previous studies Dione *et al.*, (2011) ; Fekry, Ammar and Hussien,(2018) they found *invA* gene in all *Salmonella* isolates from different sources. *InvA* is a putative inner membrane component of, essential for entry into epithelial cells, and it is a specific target gene for confirmation of *Salmonella spp*.Barilli *et al.*, (2018). This gene is present virulent strains of this microorganism, since it is one of those in charge of coding bacterial proteins that are an important part in the process of cellular invasion of the bacteria to the host. The absence of this gene in the genus *Salmonella* is rare and would determine the inability of the bacterium to invade tissues, or, that it does so by an alternative mode Sánchez *et al.*, (2019).

Simplex PCR assay, in the present study, was used to detect the presence of antibiotic resistance genes; antibiotic resistance genes used in this study were *gyrA*, *parC* and *qnrB.*

The current study showed the percentage of antibiotic-resistant genes in *Salmonella* isolated from chiken meat were: *gyrA*(93.75%), *parC* (93.75%) ,*qnrB* (6.25%), *qnrA* (0%) and *qnrS* (0%).

Fluoroquinolones kill *Salmonella spp.* by binding to DNA gyrase and causing double-stranded breaks in DNA Gopal *et al.*, (2016) .DNA gyrase consists of two A and two B subunits encoded by the gyrase A (*gyrA*) and gyrase B (*gyrB*) genes, respectively. The interaction between fluoroquinolones and DNA gyrase takes place in a conserved region of *gyrA* Kongsoi *et al.*,(2016) and *gyrB* known as the quinolone resistance-determining region (QRDR). Resistance to fluoroquinolone is frequently conferred to *Salmonella spp.* by mutations in the QRDR of *gyrA* or *gyr B*. In these organisms, resistance to fluoroquinolones has been shown to be associated most frequently with alterations in *gyrA* Macías-Farrera *et al.*,(2018).

this study presence of the *gyrA*(93.75%) in *Salmonella* from chiken meat *Salmonella* larger than result in studyCui *et al.*,(2019) was showed (64.38%) of *Salmonella* isolates positive for *gyrA* and does not correspond to a study Nakatsuchi *et al.*, (2018) shown that (88.54%) of salmonella isolates positive for *gyrA.*

Resistance to quinolone drugs is primarily mediated by mutations in (QRDR) of *gyrA* and *parC* genes in *Salmonella* and other Gram-negative organisms Eguale *et al.*, (2017). Resistance to quinolones is mainly due to: (i) mutations in (QRDRs) of the target genes *gyrA* which encode DNA gyrase, and *parC*, which encode topoisomerase IV (ii) low accumulation of the antimicrobial within the cell, mostly associated with increased efflux pump due to overexpression of the AcrAB- TolC efflux pump Lunn *et al.*,(2010)

the percentage in this study of *parC* gen (93.75%) of *Salmonella* isolates from chicken meat.this result disagree with Ball et al., 2019; Wajid et al., (2019) reported (15.6%) and (47%) isolates from from chicken farms and Poultry Farms. The discrepancy between the two studies may be due to the number of isolates investigated and the presence of multiple mutations in most of the isolates.

qnr genes These genes encode for pentapeptide proteins that protect bacterial topoisomerases from the effect of quinolones. They do not induce high level resistance but their presence leads to mutation in the QRDR Eguale *et al.*, (2017). The current study showed the presence of the *qnrB* (6.25%) in *Salmonella* from chicken were positive to *qnrB* gene . result of this study disagree with studies Saboohi *et al.*, (2012)shown (1.17%) clinical *Salmonella* isolates harbored the *qnrB* gene,while (Moawad, Hotzel and Awad, 2017)shown (20.0%) of *Salmonella* isolated from chicken meat were positive to *qnrB* gene.The lack of *qnrB* gene in the isolation of Salmonella from humans is not due to coexistence with other genes that give resistance to fluoroquinones. The emergence of these genes in chicken isolates due to the use of antibiotics in treating poultry in addition to the use of fodder that contains a high percentage of antibiotics.

This study about (0%) of human *Salmonella* isolates were negative of *qnrA* gene that disagreement with Saboohi *et al.* (2012), found (25.8%) of *Salmonella* were positive of this gene .another studies by Malehmir, Ranjbar and Harzandi, (2017) and Cattoir *et al.*(2007) were disagreement with result of this study , isolate found(16.30%) , (0.2%) *qnrA* positive,respectively.

However, results of this study shows (0%) of chiken *Salmonella* isolates were negative for *qnrA* gene,this results disagreement with study de Jong *et al.*(2014) ,that found (0.5%) of isolates were positive for *qnrA* gene . Results of this study demonstrated not found of human *Salmonella* were positive for *qnrS* gene this result don’t agree with study in Iran, Malehmir, Ranjbar and Harzandi,(2017) found (56.52%) of isolates harbored *qnrS* genes and don’t agree with study Veldman, van Pelt and Mevius (2008) in the Netherlands,found (91.17%) of *Salmonella* isolates were positive for this gene. The result of the current study don’t appear any positive results of chiken *Salmonella* isolates for *qnrS* gene ,this result agree with study Müller *et al.*(2018),found no isolate carried the gene *qnrS*, this results demonstarted don’t agree with study by Ball *et al.*(2019) that found (5.8%) of *Salmonella* were positive for *qnrS* gene.

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