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MOLECULAR CHARACTERIZATION OF GENES LINKED TO ANTIMICROBIALS AND VIRULENCE RESISTANCE IN *SALMONELLA* SP. ISOLATED FROM BROILER CHICKENS

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ABSTRACT

Fifteen *Salmonella* strains representing four *Salmonella* serovars (*S.Anatum, S.Montevideo, S.Typhimurium and S.Boecker*) were isolated, purified, and identified from freshly dead and diseased chickens suspected to be infected with Salmonellosis. In this study, antimicrobial resistance to 14 different antimicrobials and virulence genotyping to those *Salmonella* strains were performed. All isolates were completely sensitive (100%) to amikacin, Sulphamethoxazole/trimethoprim, Flumequine, Neomycin and gentamicin. Isolates responded variably to tetracycline, colistin sulfate and cephradine. Intermediate sensitivity to streptomycin and for doxycycline and Ciprofloxacin was observed.
In contrast, all isolates were completely resistant to Ampicillin, Cefotaxime and Amoxicillin. The genotypic resistance of studied serotypes against the Sulfonamide resistance gene. β-lactams resistance gene, Streptomycin resistance and Tetracycline resistance gene showed that all Salmonella studied serotypes (100%) have Sul-1gene, and were also positive for bla TEM gene, (aadA) and (TetA A) gene at PCR respectively. while two Salmonella serotypes (50%) were positive for (qnrA) gene blamable for Quinolones resistance. The efficacy of four disinfectants was evaluated; Quaternary Ammonium Compound, Iodine compound, Organic salt and Acid and H2O2 used by commercial poultry disinfectants. All the investigated 4 Salmonella serotypes showed no observable growth after treatment, with 3% hydrogen peroxide and 2% organic salt and acid and 3% QAC and visible growth at 1% and 2% hydrogen peroxide and iodine at the recommended user-dilution. Results of screening of some MDR isolates by multiplex PCR for detection of some virulence genes showed that all the tested isolates (100%) had hilA, sopB, stn, and invA.

***Keywords**: Salmonella*, invA, disinfectants, antimicrobial resistance

INTRODUCTION

Salmonella belongs to the Entero-bacteriaceae family (Brenner *et al.,* 2000). The detection of *Salmonella* in chicken farms was a serious alarm.

The combination of a sensitive culture method and a suitable sampling procedure was expected to be crucial for the success of detection (Carrique-Mas and Davies, 2008).

Both species-specific and non-species-specific *Salmonella* serovars primarily affect

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chickens as their hosts. These infections can either directly affect the health of the birds, leading to severe losses and high mortality rates in young birds, or they can make the birds more susceptible to other infections (Sedeik *et al.,* 2019; Swelum *et al.,* 2021).

ElSheikh *et al.* (2019) found that a significant number of *Salmonella* species have many virulence characteristics that contributed to their pathogenicity, controlling the mode of infection and its intensity in the intended hosts.

Antimicrobials are utilized extensively for the treatment of infections in poultry. The ongoing monitoring of antibacterial susceptibility is essential to detect shifts in the *Salmonella* resistance pattern (Kumar *et al.,* 2012).

An efficacious program for biosecurity requires the proper use of disinfectants, and an accurate implementation of disinfection procedures. This can reduce pathogens at a reasonable cost (Ahmed *et al.,* 2017).

A major risk to public health is the persistent resistance of *Salmonella* spp. to antibiotics. Additionally, virulence gene identification can aid in our understanding of *Salmonella* pathogenesis (Ghetas *et al.,* 2021).

The current work aimed to isolate and identify *Salmonella* spp. from broiler chickens, molecular detection of virulence genes and antimicrobial resistant genes in the *isolates*.

MATERIALS AND METHODS

1. Sampling

Hundred and twenty pooled samples of internal organs (yolk sac, liver, spleen, and cecum) were collected for bacteriological analysis, as described by (WHO, 2010). These samples were gathered from both dead and sacrificed. They showed symptoms in broiler chickens, between 1 and 42 days old, from different poultry farms. All the examined birds had a history of whitish diarrhea and increased flock mortalities. These specimens were collected from Assiut Governorate.

2. Biochemical Identification

Suspected colonies of *Salmonella* spp. from XLD agar were subjected to biochemical analysis using oxidase, citrate utilization test, and urease hydrolysis (Lamboro *et al.,* 2016), hydrogen peroxide and triple sugar iron agar were also tested (Barrow and Feltham 1993; MacFaddin, 2000) and fermentation of lactose using MacConkey agar

3. Serological Identification of *Salmonella* Isolates

The Animal Health Research Institute in Giza, Egypt performed serotyping of suspected *Salmonella* strains in accordance with the guidelines provided by the manufacturer, Denka Seiken Co., Tokyo, Japan.

4. Antimicrobial sensitivity test

The sensitivity of isolated *Salmonella*e to different antibacterial agents was assessed by disc diffusion test according to (EUCAST, 2015; CLSI, 2019)

5. Detecting the ability of the isolated *Salmonella* to form Biofilm

Congo-Red Agar method according to (Nachammai *et al.,* 2016)

6. Molecular analysis

6.1. Recognition of Antimicrobial Resistance Genes and Virulence Genes by PCR

The extraction of DNA from our designated isolates was conducted according to QIAamp-DNA mini-kit instructions. The Oligonucleotide Primers supplied by Metabion (Germany) are detailed in table (2)

6.2. Screening for QAC resistance gene

the qacED1 gene was detected by PCR (table 1)

Table 1: sequences of oligonucleotide primers for the studied genes

Primers	Sequences	Amplified product	Reference
SopB	"TCA GAA GRC GTC TAA CCA CTC" "TAC CGT CCT CAT GCA CAC TC"	517 bp	Huehn *et al.* 2010
QacED1	"TAA GCC CTA CAC AAA TTG GGA GAT AT" "GCC TCC GCA GCG ACT TCC ACG"	362 bp	Chuanchuen *et al.*, 2007
BlaTEM	"ATCAGCAATAAACCAGC" "CCCCGAAGAACGTTTTC"	516 bp	Colom *et al.*, 2003
AdrA	"ATGTTCCCAAAAATAATGAA" "TCATGCCGCCACTTCGGTGC"	1113 bp	Bhowmick *et al.*, 2011
TetA(A)	"GGTTCACTCGAACGACGTCA" "CTGTCCGACAAGTTGCATGA"	570 bp	Randall *et al.* 2004
InvA	"GTGAAATTATCGCCACGTTCG GGCAA" "TCATCGCACCGTCAAAGGAA CC"	284 bp	Oliveira *et al.*, 2003
Sul1	"CGGCGTGGGCTACCTGAACG" "GCCGATCGCGTGAAGTTCCG"	433 bp	Ibekwe *et al.*, 2011
Aada1	"TATCAGAGGTAGTTGGCGTCA T" "GTTCCATAGCGTTAAGGTTTC ATT"	484 bp	Randall *et al.* 2004
QnrA	"ATTTCTCACGCCAGGATTTG" "GATCGGCAAAGGTTAGGTCA"	516 bp	Robicsek *et al.*, 2006
Stn	'TTG TGT CGC TAT CAC TGG CAA CC" "ATT CGT AAC CCG CTC TCG TCC"	617 bp	Murugkar *et al.*, 2003
Hila	"CATGGCTGGTCAGTTGGAG" "CGTAATTCATCGCCTAAACG"	150 bp	Yang *et al.*, 2014

7. Disinfectant Susceptibility Testing

Two approaches were used to assess the antibacterial susceptibility of a disinfectant to determine its antimicrobial profile.

1. Disc diffusion procedure reported by (Njagi *et al.*, 2004) with a few changes. The market-available concentrations of four (4) disinfectants were utilized. Millimeters (mm) were used to measure and record each inhibitory zone. The findings elucidated along these lines; a zone from 0–5 Millimeters showed no inhibition; a zone from 6–9 Millimeters regarded medium inhibition; a zone from 10–14 Millimeters was interpreted as showing severe inhibition; and a radius of more than 15 Millimeters showed extremely strong inhibition (Daniel, 2020).

٢. In accordance with Methods of Analysis for Control of Products from Animal Origins. Dilution of the disinfecting agents made as mentioned by guidelines of the manufacturer and another different concentration table (3).

9ml of every solution of disinfectant poured in sterilized tubes, then adding bacterial colony. Afterward, the solution was added to tubes containing 10 ml RV-broth, and lastly, incubated for 24 hours at 37° C, and the presence of turbidity was detected. These samples were seeded onto Nutrient agar plates. Lack of microbial growth is decisive for the effectiveness of disinfectants (Pilotto *et al.,* 2007).

A single inoculated tube remained without disinfectants application (negative control), whereas the first dilution of every examined disinfectant expressed control positive (Al-Dabbagh *et al.,* 2015).

Table 2: Disinfecting agents with their adjusted concentrations.

Tradename	Chemical-name	Component	Concentrations
[1] Bixler	Glutaraldehyde+QAC	Glutaraldehyde+QAC	0.5% , 1%, 2%
[2] Virkon S 50%	Organic salt&Acid	Monoperoxy sulphate + Potassium peroxy Sulphate	0.5%,.1%, ,2%
[3] Sekir3%	Iodine	3% available iodine	0.5%,1%, 2% 3%
[4]H2o2	Hydrogen peroxide		10,20,30

3.1. Results of isolating and identifying *Salmonella* organisms.

3.1.1. Colonial morphology.

Using XLD-agar 90 samples showed colonies of typical *Salmonella* spp. like black-centered pink smooth colonies. However, the colonies appeared colorless, and pale using MacConkey's agar.

3.1.2. Biochemical identification

The biochemical properties of obtained suspected bacteria were typical of *Salmonella* (Table 3). Only 20 samples were confirmed to be positive by the biochemical tests.

Table 3

Biochemical reaction	TSI	Oxidase	Citrate utilization	Lysine Iron Agar	Urease	Catalase
Result	+ve Red slant /Yellow butt with H2S and gas production	-ve	+ve blue discoloration	+ve H2s production	-ve	+ve

3.1.3. Serological identification

The twenty suspected samples were serologically examined; 15 isolates were typically identified as *Salmonella* and categorized under 4 different serotypes, including *S.Typhimurium* (6), *S. Montevideo* (1), *S.Anatum* (6) and *S.Boecker* (2) (Table 4)

Table 4

Serial NO.	Serotype	O Antigens	H Antigens phase1	H Antigens phase2
1	"*S.Typhimurium*"	1, 4, [5], 12	I	1, 2
11	"*S.Anatum*"	3, [10],[15] : [15, 34]	e, h	1, 6
111	"*S.Boecker*"	[1], 6 ,14 , [25]	L, V	1, 7
1V	"S.*Montevideo*"	6, 7, 14	g, m [p], s	[1,2,7]

3.2. Biofilm formation and molecular detection of the gene responsible

All isolates were strong biofilm producers and detected the adra gene in the four isolated serotype

Figure. 1: Biofilm formed by *Salmonella*

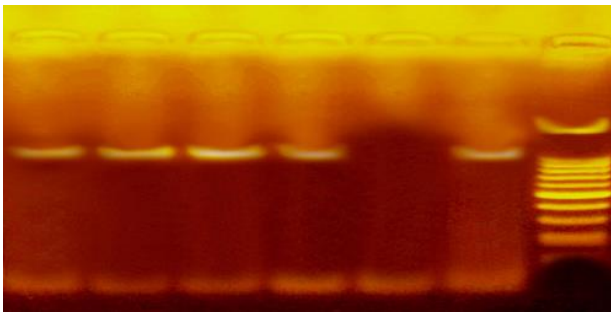


Fig. 2: Adra genes in Salmonella serotypes Positive samples

3.3. Antimicrobial Susceptibility of the isolated *Salmonella* serovars:

All isolates were completely sensitive (15/15, 100%) to amikacin (AK30), Sulphamethoxazole/trimethoprim (SXT25), Flumequine (F), Neomycin (N 10) and gentamicin (CN10).

Isolates responded variably to tetracycline (TE30) (2/15, 13.3%) and cephradine (CE) (1/15,6.6%)

They had intermediate sensitivity to streptomycin (S10) (5/15, 33.3%), and (6/15, 40%) for doxycycline (DO30) and Ciprofloxacin (cip5) (6/15, 46.6%)

All isolates were completely resistant (15/15, 100%) to Ampicillin (AMP10), Cefotaxime (CTX) and Amoxicillin (AMX 10), but they had varying resistance degrees to colistin sulfate (CL10) (2/15, 13.3%)

All fifteen isolates of *Salmonella* expressed multi-drug resistance, being resistant to at least 5 antimicrobials. 7 isolates (46,6%) were resistant to 7-8 antibiotics.

Table 5: Multi-drug resistant isolates:

Serotypes	***NO. of isolates***	***NO of Resistant antibiotics***			
		5	***6***	***7***	***8***
S.typhymurium	6	3	-	3	-
S.boecker	2	-	1	1	-
S.antum	6	1	3	1	1
S.montovido	1	-	-	1	-
Total no		5	4	6	1

3.4. Molecular antibacterial resistance profile of studied *Salmonella* serotypes :

1. Sulfonamide resistance (Sul-1) gene β-lactams resistance (bla TEM) gene, Streptomycin resistance (aadA) and Tetracycline resistance (TetA A) gene.

All *Salmonella* studied serotypes (4/4) (100%) involved Sul-1gene; giving its characteristic 433 gene fragment (Fig. 3) also were positive for bla TEM gene, which gave 516bp fragments at PCR (Fig. 3) and positive for (TetA A) and (aadA) gene, giving 570 bp and 484bp fragments at PCR, respectively.

2. Quinolones resistance (qnrA) gene

Two *Salmonella* isolated serotypes (2/4) (50%) were positive for gene (qnrA) among 4 inspected *Salmonella* serotypes that produce amplification at 516bp fragment Fig. (3).

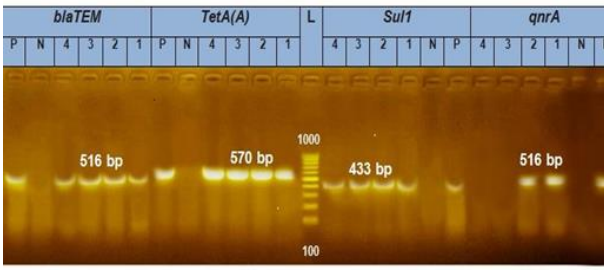


Fig. 3: Sul – 1,bla TEM and TetA A genes for Salmonella serotypes (+)ve results showed band (433;516; 570 bp), respectively. While gyrA gene was positive in 2 serotypes producing band (516) bp.

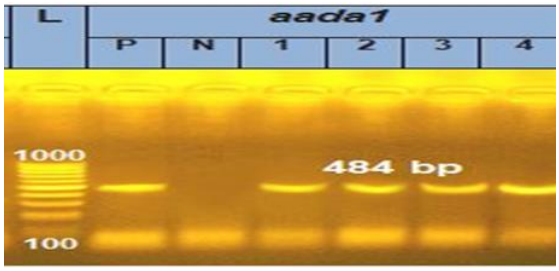


Fig. 4: aada1 genes in Salmonella serotypes Positive samples produce band 484 bp).

3.5. Evaluation of disinfectants against *Salmonella*

3.5.1. Disinfectant Susceptibility Profiles of *Salmonella* serotypes

In general, disinfectants displayed varied degrees of activity toward the four *Salmonella* serotypes.

Table 6:

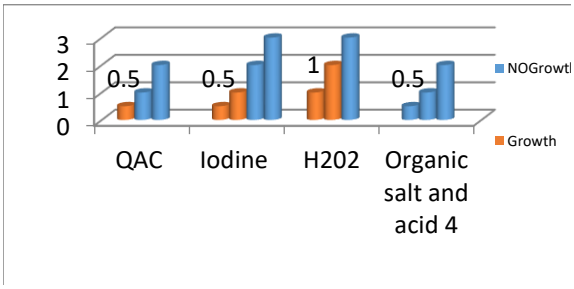
Disinfectant	Organic salt&Acid			Hydrogen peroxid			QAC			Iodine			
Concentration	**.5%**	**1%**	**2%**	**1%**	**2%**	**3%**	**.5%**	**1%**	**2%**	**.5%**	**1%**	**2%**	**3%**
S.serotype													
S.typhimurium	9mm	10mm	20mm	0	0	22mm	0	15mm	17mm	0	4mm	6mm	15mm
S. anatum	8mm	15mm	16.5mm	0	2.5mm	17.5mm	4.5mm	7mm	17mm	0	5mm	6.5mm	16mm
S.boecker	2.5mm	15.5mm	19.5mm	0	4mm	18.5mm	4mm	14.5mm	16mm	0	0	0	7.5mm
S. montevideo	5mm	15mm	17.5mm	0	6mm	17mm	0	0	15mm	0	4mm	12.5mm	16.5mm

Clearly, in the S. serotypes under study, 3% H2O2 showed the biggest inhibitory radius, measuring between 17 to 22 millimeters radius, indicating the widest range of antibacterial action.

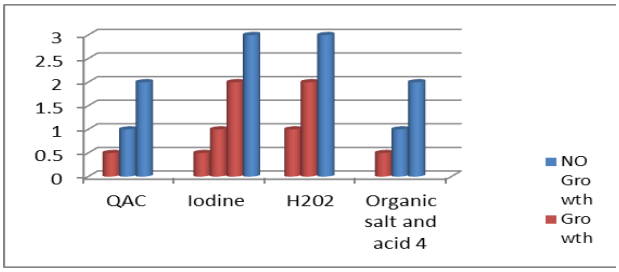
Inhibitory activity of 3% hydrogen peroxide and 2% Organic salt and Acid and 3% QAC conc. was obvious. All serotypes are completely resistant to 1% H2O2 and iodine using the manufacturer's instructed dilutions. 1% QAC and 1% Organic salt and Acid showed moderate to strong antibacterial activity. However, QAC and Organic salt and Acid at user-recommended concentration and 2% iodine showed moderate to no growth inhibition.

3.5.2. Using the technique of Analysis for Control of Products of Animal Origin (Pilotto *et al.*, 2007).

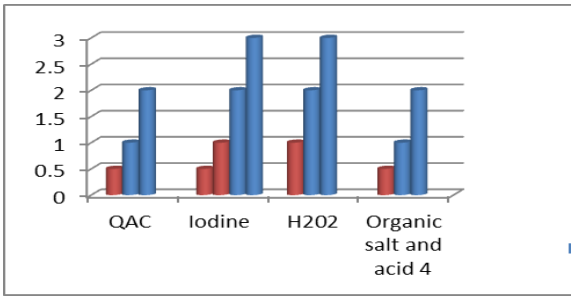
The four isolated *Salmonella* serotypes showed no observable growth after treatment with 3% hydrogen peroxide, 2% Organic salt and Acid and 3% QAC. Visible growth at 1% and H_2O_2 and iodine at the user-recommended dilution.



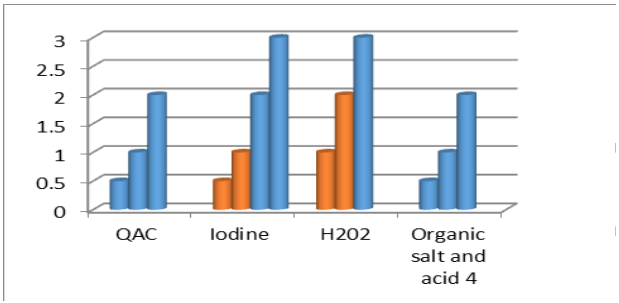
Disinfectant efficacy against S. *Typhimurium*



Disinfectant efficacy against S. *boecker*



Disinfectant efficacy against *S.anatum*



Disinfectant efficacy against *S. montevedo*

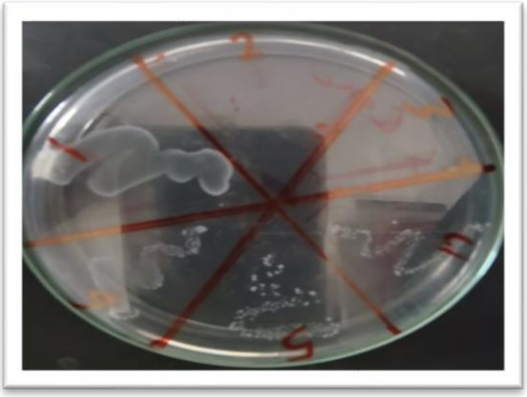


Figure 5: show disinfectant susceptibility test of (H_2O_2) (1) as C +ve, (2) as C -ve, (3) 1% H_2O_2, (4) 2% H_2O_2, (5) 3% H_2O_2

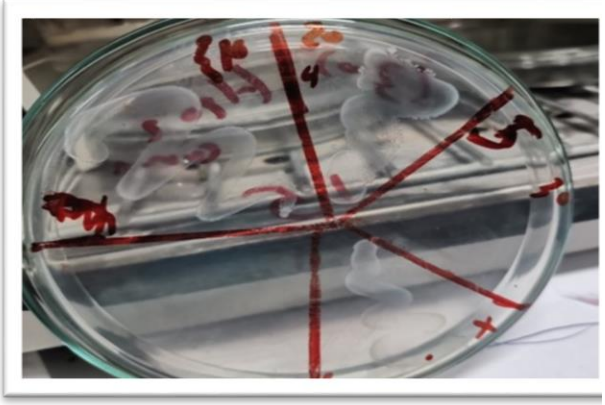


Figure 6: Show disinfectant susceptibility test (iodine) of *Salmonella boecker* (1) as a positive control, (2) as a negative control, (3) 3% iodine, (4) 2% iodine, (5) 1% iodine, (6), 5% iodine

From Figure (5), we observe growth at No. 3,4 and (1%, 2% H_2O_2), and no growth at No.3 (3% H_2O_2)

From Figure (6), we observe growth at No. 4, 5 and 6 (.5%, 1%, 2% iodine) and no growth at No.3 (3% iodine)

3.6. Genotypic outcome of resistant QAC.

The results showed that the four examined isolates' serotypes contain the qacED1 gene (Fig.7) .

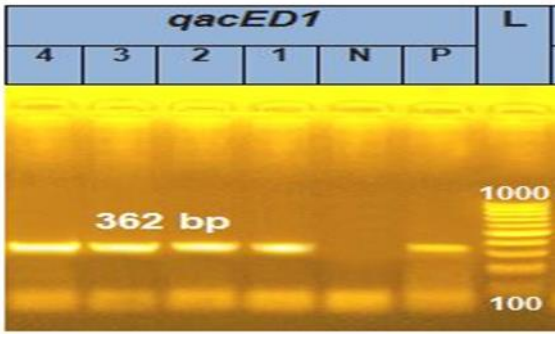


Figure 7: qacED1 +ve in 4 *Salmonella* isolates produce band 362 bp and the positive control lane

3.7. Molecular examination for genes of virulence of the *Salmonella* isolate :

The incidence of hilA, stn, sopB, invA was 100% (Fig8.)

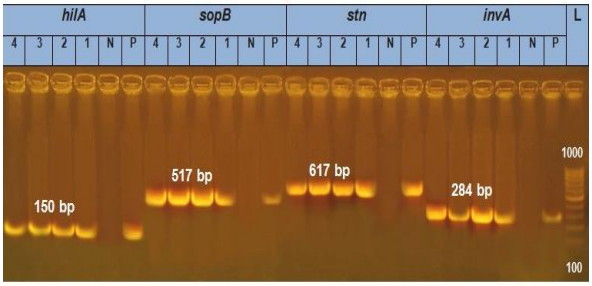


Figure 8: All identified virulence genes (invA, hilA, sopB and stn) in studies of *Salmonella* serotypes were positive samples producing band 284 pd, 150 pd, 517 pd and 617 pd), respectively.

DISCUSSION

Depending on the conventional bacterio-logical examination and biochemical tests, the typical *Salmonella* colony in this study was isolated from 15 samples (12.5%) of 120 pooled examined chicken samples from different poultry farms. These birds suffered from signs of septicemia, a history of whitish diarrhea and dead broiler chickens. These results are in line with Rihan, (2004) who reported a 12% prevalence of *Salmonella* in broilers, and by Lebdah *et al.* (2017) reported a *Salmonella* prevalence (10.7%) in chicken in Dakahlia, Damietta, Gharbia, Kafr-Elshikh and Sharkia Governorates, and Mahmoud and Mousa (2000), Verma *et al.* (2000) and EL-Mohsen *et al.* (2022) stated the *Salmonella* occurrence 9.17, 15.02, 11.35%, respectively.

Ibrahim *et al.* (2018) recorded a *Salmonella* prevalence of 6.8% in broilers in Behaira. While Helal *et al.* (2019) recorded 5.33% in El-Minufyia and El-Gharbia governorates, in Egypt. Sedeik *et al.* (2019) 7.5% in newly hatched chicks in five different governorates in Egypt.

The geographical dispersion of samples, management practices, type of birds, biosecurity, and the preventive antimicrobials employed in each situation might all be responsible for these variations in the results (WHO, 2007).

The characteristic colony for isolated *Salmonella* spp. As described by El-Tawab *et al.* (2015).

Biochemically, the results of the obtained 15 isolated *Salmonella* matched (Hossain, 2002; Issa *et al.* 2017).

Many different *Salmonella* serovars can infect poultry. The most frequently reported serovars worldwide were found to be *S. typhimurium* and *S. enteritidis* (WHO, 2007).

In this study, the four encountered *Salmonella* serovars were identified as *S.typhimurium* (6) (40%), *S.anatum* (6) (40%), *S.boecker* (2) (13.3%) and *S. Montevideo* (1) (6.6%).

Our result to some extent agrees with lebdah *et al.* (2017), who identified sixteen strains among several Egyptian localities, where *S.typhimurium* considered the predominated serotypes. Furthermore, according to Kaoud *et al.* (2018), *S.typhimurium* (25.71%) ranked as the most prevalent serotype in broilers, having been recovered from chicken in several Egyptian governorates. Additionally, *S. typhimurium* (52.94%) and *S. enteritidis* (11.76%) are the two most common serotypes in Egypt (Ammar *et al.*, 2016).

S. *montevideo* (86 incidents; 21.0% of all incidents) was the most commonly reported serovar for all *Salmonella* serovars isolated from hens between 2007 and 2011, and the number of reports of *S. montevideo* has been rising quickly in the last few years. Although there were just two *S. montevideo* occurrences recorded in 2008, the number

increased to 13 in 2009, 49 in 2010, and 86 in 2011 (Gorski *et al.* 2022).
Although the cause of this increase is unknown, it is believed to be related to contaminated feed ingredients, because there has been a noticeable rise in this serovar in feeding materials. Also holding the same opinion of Papadopoulou *et al*. (2009)

Salmonella montevideo was found in notable clusters, suggesting that the bacteria entered one or more flocks at a definite point in time (for example, by food contamination and also infection to newly hatched chicks).

In seven governorates in Egypt, Rabie *et al.* (2023) isolated *S. typhimurium* (21.2%) and *S. Montevideo* (9.1%) from broiler chickens.

Salmonella anatum represented as the second serovars identified in the current investigation with high prevalence. These data, to some extent, matched those of Rodríguez *et al.* (2006), who isolated *Salmonella anatum* with a percentage of (48.4%) in poultry farms, and also Lamas *et al*. (2016), with the incidence of 14.92% in broiler flocks.

According to the results of antimicrobial susceptibility, fifteen *Salmonella* isolates displayed complete resistance to Ampicillin (AMP10), Amoxicillin (AMX 10) and Cefotaxime (CTX), followed by streptomycin (93.3%), Tetracycline (86.6%), Cephradin (80%) and Ciprofloxacin (64.6%). All isolates of *Salmonella* in this work harmonized those of Rahman *et al.* (2016), confirming amoxicillin resistance. High ampicillin resistance is common in *Salmonella*, more or less in agreement with Zhao *et al.* (2017) mentioning high resistance against ampicillin (69.4%) in *Salmonella* isolates

Our isolated serotypes were completely susceptible to amikacin (AK30), Sulphamethoxazole-trimethoprim (SXT25), Flumequine (F), Neomycin (N 10) and gentamicin (CN10). followed by Colistin sulfate (86.6%), Doxycycline (40%). Our findings were in line with Ezzat *et al.* (2019): Abou Zeid *et al.* (2020) described that *Salmonella* isolates were completely sensitive to amikacin, which was the ideal chemotherapeutic drug for treating Salmonellosis, following that sulphamethoxazole-trimethoprim (50%) also gentamicin (50%). *Salmonella* isolates were noticed by Hasan *et al.* (2018) to be highly susceptible to doxycycline, and gentamicin. Elshebrawy *et al.* (2021), on the other hand, discovered that 62.8% of the isolated *Salmonella* exhibited extreme resistance to trimethoprimsulphame-thoxazole and amikacin. Furthermore, the results of Abdeen *et al.* (2018) in Egypt declared that gentamicin resistance rate was (25%).

Doxycycline resistance was recorded in this study (20%) which is lower than the resistance (96 %) obtained by Al-baqir *et al.* (2019). In addition, our results disagree with Moe *et al.* (2017), who found all isolated *Salmonella* showed resistance to ampicillin (47.1%), tetracycline (54.3%), ciprofloxacin (9.4%), gentamicin (8%), and sulfametho-xazole/trimethoprim (70.3%).

All fifteen isolated *Salmonella* were multi-drug resistant to at least 5 antimicrobials. Seven isolates (46, 6%) were resistant to 7-8 antibiotics.

These results were closely similar to Abdeen *et al.* (2018), who mentioned that all studied isolates of *Salmonella* were multidrug resistant for 5 antimicrobials. These results agree with Al-baqir *et al.* (2019) in Egypt, who spotted multi-resistant drugs for isolated *Salmonella* in at least four antimicrobials, while 49 % of *Salmonella* isolates were resistant to eight: nine antimicrobials and more or less are in accordance with these reported by Ammar *et al.* (2016) 52.94%, Khallaf *et al.* (2014) 42.1% in Morocco, and according to Sharma *et al.* (2019), 92.86% of the isolates demonstrated resistance to five or more antimicrobials using antibiotic sensitivity test, indicating that all *Salmonella* isolates were multi-drug resistant.

PCR is a method for precisely detecting genes resisting antibiotics of *Salmonella*. The gene sul-1, which codes sulfa-drugs resistant, was 100% in the identified serotypes in this study. Our findings are consistent with (Niu *et al.,* 2020) that identified gene sul-1 at 97.8%. While Abd El-Tawab *et al.* (2015) had lower findings with 87% sul-l.

Resistance of ampicillin in the isolated *Salmonella* srevoars was confirmed by the existence of blaTEM (100%). Our findings agreed with the outcomes previously mentioned by Lu Y *et al.* (2011) and Badr *et al.* (2021). They noticed that bla TEM gene in 100% of isolates. However, our findings exceeded those reported by Abd ElTawab *et al.* (2015) publicized 69.4% for bla-TEM.

In this work, the four *Salmonella* serotypes had an encoded gene for tetracycline resistance (TetA genes) with a percentage of 100%, which to less extent agrees with (Zishiri *et al.,* 2016), who detected TetA genes in (83%) of the Brazilian broiler chicken isolates.

In our work, we found that 100% of the aadA genes, encoded for resistance of streptomycin, the present findings been superior to those from (Chen *et al.,* 2020) documented aadA1 by 83.8%. Also, El-Sharkawy *et al.* (2017) detected streptomycin resistance aadA1 (50%) in *Salmonella* isolated from Egypt farms.

Fluoroquinolones broadly consumed in poultry to fight pathogenic bacteria all over the world. This study found that 50% of the tested serotypes had plasmid-mediated quinolone resistance genes (qnr), which is consistent with the findings of Shaaban *et al.* (2015) that most *Salmonella* isolates were found to carry quinolone resistance genes. It was discovered that the –ve gm bacteria isolated from poultry farms in Egypt have qnr of 6.9% (Ishida *et al.,* 2010).

According to Niu *et al.* (2020), different conditions and antibiotic dosages used in farms, which can affect the detection rates of resistant genes. This suggests that several genes linked to bacterial-resistant antibiotics are silenced in vitro, but they can become active in vivo when antibiotic pressure is applied, This agrees with past studies by Ma *et al.* (2007).

In this study, all *Salmonella* serotypes have been sensitive to sulphamethoxazole /trimethoprim although they have gene of sul-1, this is attributed to the silence of genes responsible for resistance in isolates of *Salmonella* (Chen *et al.,* 2020).

Using polymerase chain reaction technique to identify four virulence genes in four *Salmonella* isolates. The findings showed whole investigated isolates have the sop B, hilA, stn, invA genes.

Regarding our outcomes of invA-gene, related discoveries matched with previous Egyptian works (Awad *et al.,* 2020; Hassan *et al.,* 2021). In other investigations, the results of the STN virulence genes also in line with the work of ElSheikh *et al.* (2019); Hassan *et al.* (2021).

We screened the 4 serotypes for the sopB gene, and the obtained data have been recognized by 100% in our studied serotypes it agrees with Rahman, (2006) who reported sop B gene was detected in all the examined isolates in South Asia and disagree with Ammar *et al*. (2016) reported sop B in 41.18% of the isolates and also reported hilA gene located (88.24%) in *Salmonella* isolates it near similar with our result In the contrast with earlier results, hilA was found in every isolated *Salmonella* from chicken (Cardona-Castro *et al.,* 2002; Crăciunaş *et al.,* 2012)

Salmonella forms biofilms on biological and non-biological surfaces (Solano *et al.,* 2002). The primary constituents of biofilm are cellulose, while other elements, such as curli, which give rise to the many morphotypes that are visible on Congo red agar. The morphotype known as rdar, which stands for red, dry, and rough, is distinguished by the

cellulose formation and amyloid fibers (Chia *et al.,* 2011). The csgD and adrA genes, which control and express cellulose, respectively, are strongly linked to this morphotype (Fabrega and Vila 2013). Antimicrobial agents and disinfectants can no longer effectively combat biofilm bacteria (Wong *et al.,* 2010).

Because biofilms make bacteria more resilient to stress, they are less susceptible to the effects of antibiotics and disinfectants (Sereno *et al.,* 2017). The present findings evidenced every *Salmonella* e isolate may produce biofilm in 24-48 hours. This is a warning of public health hazards. We detected the presence of the adrA genes responsible for biofilm formation via polymerase chain reaction in every examined serotype similar findings were obtained by Seixas *et al.* (2014); and Abd El-basit *et al.* (2019). While Lamas *et al.* (2016) declared that 80.82% of examined samples were positive for adrA gene

McLaren *et al.* (2011), illustrated that disinfection can only be successful if the proper disinfectant is applied at the proper concentration. Moreover, Randall *et al.* (2004) explained that Frequent usage and extended application of disinfecting agents can cause *Salmonella* to become resistant, reducing their efficacy.
At present, we have a lack of research on the in vitro effectiveness of marketed disinfecting agents on *Salmonella* (Jang *et al.,* 2017; Drauch *et al.,* 2020).

The purpose of this work was to examine the efficacy of frequently applied disinfecting agents on *Salmonella* isolated serotypes taking into account, disinfectant concentration.

Findings of the disinfectant sensitivity test revealed the whole isolated serotypes were sensitive to 3% Hydrogen peroxide, 2%QAC then 3% Iodine this agrees with Aksoy and Yardimci, (2020) reported that H_2O_2 was the most effective disinfectant then iodine against avian *Salmonella*e.

De Quadros *et al.* (2020) demonstrated the effectiveness of peroxygens against several isolates of *Salmonella*, This agrees with the present findings, Miguel Ruano *et al.* (2001) declared 2% conc. of H_2O_2 had great action against microbes. The genetic differences between *Salmonella* serotypes obtained from various hosts or geographical regions might be the cause of this disparity (Antony *et al.,* 2018).

Our serotypes are completely resistant to 1% H_2O_2 and iodine at the suggested manufacturer's instructed dilution but with increasing the concentration of disinfectant it showed moderate to strong antibacterial activity this result is in line with findings from Aksoy *et al.* (2020) who stated the disinfecting agents works better against microorganisms by raising their concentrations.

Properly examination of disinfecting agent conc. and formulations are necessary (Miguel Ruano *et al.,* 2001). As an illustration, It is common to suggest and utilize organic salt, acid, and QAC for poultry farming at.5% conc, However, in the current study this concentration had produced unsatisfactory findings. Since QAC has been utilized in the chicken industry for a long time, this is not shocking. Extended usage of disinfecting agents may attribute to developed resistance.

Our result is in agreement with the outcomes of Gilinsky, (2006), and Gehan *et al.* (2009), who observed varying degrees of bacterial susceptibility to quaternary ammonium compounds particularly for S.typhimurium.

The QAC genes are extensively distributed in both environmental and clinical bacteria and this spread is typically related to specific types of bacteria (Jaglic and Cervinkova 2012). The current work identified the qacED1- gene by PCR in 100% representative isolated *Salmonella* serotypes, our findings were compatible with Bakheet *et al.* (2017), who explains that there is a substantial correlation between the

existence of qacED1 and the resistance of antimicrobes.

Interestingly, every isolate of *Salmonella* spp. that tested positive for qacED1 in their investigation was resistant to multiple drugs and partially agreed with Asherf *et al.* (2016) reported 57.14% of *Salmonella* samples had the qacED1 gene, our findings were almost identical to those of Amira, (2016), that discovered that qacED 1 was distributed with a 93.1%. The findings matched those of Wang *et al.* (2008), who assumed that negative gram microorganisms, primarily belonging to the Enterobacteriaceae family, possess a wide distribution of the qacE gene. They further conjectured that disinfectant resistance could potentially contribute to antibiotic resistance through co-selection or co-resistance mechanisms.

CONCLUSION

This study isolated 15 isolates that included four different serotypes, which showed multi drug resistant. The frequent utilization of antimicrobials in the Egyptian broiler farmhouses triggering higher MDR value. Regular estimation of the antibacterial sensitivity of the most common strain of *Salmonella* is required. Our isolated serotypes were resistant to different disinfectants in their recommended doses. The disinfectants were effective only after increasing their concentration. Therefore, it is advisable to re-estimate the recommended disinfectant doses used in the poultry field.

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الخصائص الجزيئية المرتبطة بجينات الضراوة ومقاومة المضادات الميكروبية لعدوى السالمونيلا في دجاج التسمين

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تم عزل١٥ معزولة من السالمونيلا والتي اشتملت على اربعة سلالات واظهرت البكتريا المعزولة مقاومة عالية للمضادات الحيوية والمطهرات المستخدمة في هذه الدراسة، تم إجراء اختبار حساسية المضادات الحيوية باستخدام ١٤ نوعًا مختلفًا من المضادات الحيوية. كانت جميع المعزولات حساسة تماماً (١٠٠%) لكل من الأميكاسين، السلفاميثوكسازول/تريميثوبريم، الفلومكين، النيومايسين والجنتاميسين. استجابت المعزولات بشكل متفاوت للتتراسيكلين الكوليستين والسيفرادين. وقد لوحظت حساسية متوسطة للستربتوميسين والدوكسيسيكلين والسيبروفلوكساسين. وعلى النقيض، كانت جميع العزلات مقاومة تمامًا لمضادات الأمبيسلين والسيفوتاكسيم والأموكسيسيلين. وبمعالجة السلالات المعزولة باستخدام اربعة مطهرات بتركيزاتها المختلفة وهما مركب الأمونيوم الرباعي ومركب اليود والفيركون اس والبيروكسد هيدروجين، أظهرت معزولات السلمونيلا التي تم فحصها عدم وجود نمو ملحوظ بعد المعالجة بـ ٣% بيروكسيد الهيدروجين و٢% فيركون اس و٣% QAC ونمو ملحوظ عند استخدام تركيز ١% و٢% بيروكسيد الهيدروجين واليود وهذا هو التخفيف الموصى به من قبل المنتج. وبناءا علي ذلك وجد ان فاعلية المطهرات تزداد بزيادة تركيزها. باستخدام تقنية تفاعل البلمره المتسلسل (PCR) اثبت ان جميع السلالات المعزوله تحتوي علي جينات qacED1, adra, sop , stn , invaالمسئولة عن ضراوته العالية. وتوصي هذه الدراسة بتقييم فاعلية المطهرات لاستخدام التركيزات المثلى لها واستخدام المضادات الحيوية الاكثر فاعلية لمواجهة الاصابة بالسالمونيلا.