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Original Research Article

Genetic analysis of multidrug resistant *Salmonella* isolated from broiler chickens

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ABSTRACT

Salmonellosis is a major problem for the poultry industry, and this problem represents a critical food safety hazard. Resistance to antimicrobial agents within nontyphoidal Salmonellae is a serious problem. The present study aimed to analyze genetically some β-lactamase resistance genes and some virulence associated genes in Salmonella isolates from broiler chicken. Five hundred samples were collected from diseased broiler chickens of different ages (3-6 weeks) from different farms in Assiut Governorate in Egypt during the period from January 2015 to December 2015. Bacteriological examination showed that 26 Salmonella isolates were recovered with a prevalence rate of 5.2% Serotyping of Salmonella isolates showed that S. Enteritidis S. Typhimurium, and S. Kentuky were identified at rates of 50%, 30.8% and 19.2%, respectively. Results of antibiogram showed that 18 Salmonella isolates (92.3%) were multidrug resistant. All isolates were screened for the presence of 2 β-lactamase resistance genes (blaCTX and blaCMY) as well as 3 virulence genes (stn, invA and hilA) using multiplex PCR. The overall prevalences were 53.9% for blaCTX and 34.6% for blaCMY. Meanwhile, stn, invA and hilA genes were found in 96.2%, 100% and 84.7% of isolates, respectively.

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1. Introduction

Salmonellosis is a major problem for the poultry industry, and this problem represents a critical food safety hazard. In addition, there are reports of non-typhoidal serovars causing salmonellosis in chickens (**Gong *et al.*, 2016**).

Although more than 2500 serotypes of *Salmonella* have been identified, in recent years, *S. enterica* serovar Enteritidis (*S.* Enteritidis) and *S. enterica* serovar Typhimurium (*S.* Typhimurium) have been recognized as the two major causative agents of salmonellosis in birds, mammals and humans (**Darwin and Miller, 1999**).

Although antimicrobials are valuable tools to treat clinical disease and to maintain healthy and productive birds, antimicrobial drug use has been implicated as a risk factor in the development and dissemination of drug resistance **(Gosh and LaPara, 2007)**. Food of animal origin and their production environments are reservoirs of both resistant bacteria and resistance genes that could be transferred to humans either by direct contact or indirectly via the food production chain **(WHO, 2011)**. Therefore, the appropriate antibiotic should better be selected on the basis of its sensitivity which could be detected by laboratory examination. The recovery of antimicrobial-resistant *Salmonella* in foods of animal origin has raised concerns that the treatment of human salmonellosis may be compromised because antimicrobial-resistant strains appear to be more often associated with severe disease than are susceptible isolates.

Resistance to antimicrobial agents within nontyphoidal Salmonella serotypes is considered a serious problem worldwide. Surveillance data demonstrates an obvious increase in overall antimicrobial resistance among salmonellae from 20-30% in the early 1990s to as high as 70% in some countries in the 2000s (**Su *et al.*, 2004**). More than 340 beta-lactamases have been described in *Salmonella* and the prevalence of genes encoding for them varies region by region (**Hasman *et al.*, 2005**).

The presence of several virulence genes has been positively linked to the pathogenicity Salmonellae **(Radwan *et al.*, 2016)**. The majority of the *Salmonella* isolates (99.3%) from human and food origin carried the *invA, mgtC, stn, sopB, sopE1* and *sefA* virulence genes (**Zou *et al,* 2012**). The establishment of PCR assays was to facilitate determination of the frequency with which the various virulence-associated genes occur in the resident Salmonellae populations.

The purpose of this study was genetic analyses of some β-lactamase resistance genes (bla_{CTX} *and* bla_{CMY}) as well as some virulence associated genes (*stn, invA and hilA*) in Salmonellae isolates from broiler chicken.

2. Material and methods

2.1. Samples

Five hundred samples were collected from diseased broiler chickens (suspected to harbor *Salmonella*) of different ages (3-6 weeks) from different farms in Assiut Governorate in Egypt during the period from January 2015 to December 2015. These chickens were subjected to clinical and postmortem examinations. Samples were collected aseptically from the lesions in the internal organs including liver, heart, lung, air sacs, and kidney.

2.2. Isolation and identification of Salmonella species

Samples were cultured into selenite-F broth and incubated at 37C for 18-24 hrs. Then, a loopful of this culture was streaked out onto MacConkey's agar then the non-lactose fermenter (pale) colonies were streaked onto xylose lysine deoxycholate (XLD) and Salmonella-Shigella (SS) agar media and incubated at 37C for 18-24 hours. All isolates were identified as *Salmonella* species based on their colony morphology and biochemical tests according to schemes described by **Collee *et al.* (1996)** and **Quinn *et al*. (2002)**. The *Salmonella* isolates were also confirmed biochemically by using the API 20E system (BioMérieux, Marcy-l'Étoile, France).

2.3. Serotyping of Salmonella species

Salmonella isolates were serotyped by slide agglutination test carried out according to Kauffman-White scheme **(Kauffman, 1974)** for the determination of Somatic (O) and flagellar (H) antigens using Salmonella antiserum (DENKA SEIKEN Co., Japan).

2.4. Antimicrobial susceptibility testing

All Salmonella isolates were tested for their antimicrobial susceptibility to 14 different antimicrobial discs including erythromycin (15µg), nalidixic acid (30µg), penicillin (10 IU), amoxicillin (30µg), oxytetracycline (30µg), sulphamethoxazole-trimethoprime (25µg), ampicillin (10µg), streptomycin (10µg), neomycin (30µg), chloramphenicol (30µg), norfloxacin (10µg), ciprofloxacin (5µg), kanamycin (30µg) and gentamicin (10µg) (Oxoid Limited, Basing Stoke,

UK). Antimicrobial susceptibility testing was performed using disc diffusion method on Muller Hinton agar according to **CLSI (2015)**. The antimicrobial susceptibility was based on the induced inhibition zones according to the guidelines of the **CLSI (2015)**. Resistance to two/or more antimicrobials of different classes was taken as multidrug resistant (MDR) **(Chandran *et al*., 2008)**.

2.5. Multiplex-PCR for detection of β-lactamases resistance and virulence genes

DNA was extracted by using bacterial DNA extraction kits (Qiagen, Germany, GmbH) according to the manufacturer instructions. The multiplex-PCR assay was applied on 26 Salmonella isolates for detection of 2 β-lactamase resistance genes bla_{CTX} (responsible for cefotaxime resistance) and bla_{CMY} (responsible for cephalosporin resistance) as well as 3 virulence associated genes (*stn, invA and hilA*). Targeted genes and their primer sequences are listed in (Table 1).

Table (1). Primer sequences and amplified products for the β-lactamase resistance and virulence genes.

Genes			Primer Sequence5'-3'	Amplified product	Reference
Resistance	Bla_{CTX}	F	CGCTTTGCGATGTGCAG	550 bp	Ahmed *et al.* (2009)
		R	ACCGCGATATGCTTGGT		
	Bla_{CMY}	F	GACGCCTCTTTCTCCACA	1007 bp	
		R	TGGAACGAAGGCTACGTA		
Virulence	*invA*	F	GTGAAATTATCGCCACGTTCGGGCA	284 bp	Shanmugasamy *et al.* (2011)
		R	TCATCGCACCGTCAAAGGAACC		
	stn	F	CTTTGGTCGTAAAATAAGGCG	260 bp	Makino *et al.* (1999)
		R	TGCCCAAAGCAGAGAGATTC		
	hilA	F	CTGCCGCAGTGTTAAGGATA	497 bp	Guo *et al.* (2000)
		R	CTGTCGCCTTAATCGCATGT		

1. Results

1.1. Bacteriological isolation of *Salmonella* species.

Out of 500 broiler chicken samples, 26 *Salmonella* isolates were recovered with a prevalence rate of 5.2%.

1.2. Serotyping of *Salmonella* isolates.

Three serotypes were identified among 26 *Salmonella* isolates as *S.* Enteritidis (13/26), *S.* Typhimurium (8/26) and *S.* Kentuky (5/26) with prevalence rates of 50%, 30.8% and 19.2%, respectively.

1.3. Antimicrobial susceptibility testing.

Results of *in-vitro* sensitivity tests illustrated in (Table 2) showed that *Salmonella* isolates were completely resistant to erythromycin and penicillin while they were highly resistant to amoxicillin and streptomycin (92.3% for both), nalidixic acid (80.8%), sulphamethoxazole-trimethoprime (76.9%), ampicillin (69.2%) and oxytetracycline (65.4%). On contrary, *Salmonella* strains were highly sensitive to kanamycin (96.2%) then gentamycin (73.1%). MDR *Salmonella* isolates were 24 isolates (92.3%).

Table (2): Distribution of Antimicrobial susceptibility for Salmonella isolates (*n=26*).

Antimicrobial agent	Sensitive		Intermediate		Resistant	
	NO.	%	NO.	%	NO.	%
Erythromycin (E)	-	-	-	-	26	100
Penicillin(P)	-	-	-	-	26	100
Amoxicillin (AMX)	-	-	2	7.7	24	92.3
Streptomycin (S)	2	7.7	-	-	24	92.3
Nalidixic acid (NA)	2	7.7	3	11.5	21	80.8
Sulphamethoxazol-trimethoprime (SXT)	4	15.4	2	7.7	20	76.9

Ampicillin (AMP)	**5**	**19.2**	**3**	**11.5**	**18**	**69.2**
Oxytetracycline (T)	**5**	**19.2**	**4**	**15.4**	**17**	**65.4**
Chloramphenicol (C)	**7**	**26.9**	**6**	**23.1**	**13**	**50.0**
Norfloxacin (NOR)	**11**	**42.3**	**5**	**19.2**	**10**	**38.5**
Neomycin (N)	**13**	**50.0**	**7**	**26.9**	**6**	**23.1**
Ciprofloxacin (CIP)	**14**	**53.8**	**8**	**30.8**	**4**	**15.4**
Gentamycin (G)	**19**	**73.1**	**5**	**19.2**	**2**	**7.7**
Kanamycin (K)	**25**	**96.2**	**-**	**-**	**1**	**3.8**

%: calculated according to the No. of isolates (*n= 26*)

1.4. Multiplex-PCR for detection of β-lactamases resistance genes (*bla_{CTX} and bla_{CMY}*).

Multiplex-PCR assay was done for 26 salmonella strains for detection of 2 β-lactamase resistance genes *bla_{CTX}* and *bla_{CMY}*. The results revealed that *bla_{CTX}* gene was found in 14/26 isolates (53.9%) arranged as follow; 8 *S. Enteritidis* (30.8%), 4 *S. Typhimurium* (15.4%) and 2 *S. Kentucky* (7.7%). Meanwhile, *bla_{CMY}* gene was found in 9/26 isolates (34.6%) arranged as follow; 4 *S. Enteritidis* (15.4%), 3 *S. Typhimurium* (11.5%) and 2 *S. Kentucky* (7.7%) (Tables 3 & 4 and Fig. 1 & 2).

Table (3): Multiplex-PCR results for β-lactamase resistance and virulence genes in *Salmonella* isolates.

NO	Serotype	Resistance genes		Virulence associated genes		
		bla_{CTX}	*bla_{CMY}*	*stn*	*invA*	*hilA*
1	***S. Enteritidis***	+	-	+	+	+
2	***S. Enteritidis***	+	+	+	+	+
3	***S. Enteritidis***	-	-	+	+	+
4	***S. Enteritidis***	-	-	+	+	-
5	***S. Enteritidis***	+	-	+	+	+
6	***S. Enteritidis***	-	-	+	+	+
7	***S. Enteritidis***	+	-	+	+	+
8	***S. Enteritidis***	+	+	+	+	+
9	***S. Enteritidis***	+	+	+	+	+
10	***S. Enteritidis***	+	-	+	+	-
11	***S. Enteritidis***	-	-	-	+	+
12	***S. Enteritidis***	-	+	+	+	+
13	***S. Enteritidis***	+	-	+	+	-
14	***S. Typhimurium***	+	-	+	+	+
15	***S. Typhimurium***	-	+	+	+	+
16	***S. Typhimurium***	-	-	+	+	+
17	***S. Typhimurium***	+	-	+	+	+
18	***S. Typhimurium***	+	+	+	+	+
19	***S. Typhimurium***	-	+	+	+	+
20	***S. Typhimurium***	+	-	+	+	+
21	***S. Typhimurium***	-	-	+	+	+
22	***S. Kentucky***	-	+	+	+	+
23	***S. Kentucky***	-	-	+	+	+
24	***S. Kentucky***	+	-	+	+	+
25	***S. Kentucky***	-	-	+	+	-
26	***S. Kentucky***	+	+	+	+	+
	Total (26)	**14 (53.9%)**	**9 (34.6%)**	**25 (96.2%)**	**26 (100%)**	**22 (84.6%)**

%: calculated according to the Total No. of isolates (*n= 26*)

Table (4): Resistance phenotype and prevalence of β-lactamase resistance genes in *Salmonella* isolated from diseased broilers.

NO	Serotype	Resistance phenotypes	Resistance gene
1	*S. Enteritidis*	E, P, AMX, S, NA, SXT, AMP, T, C, NOR, N, CIP, G	bla_{CTX}
2	*S. Enteritidis*	E, P, AMX, S, NA, SXT, AMP, T, C, NOR, N	bla_{CTX}, bla_{CMY}
5	*S. Enteritidis*	E, P, AMX, S, NA, SXT, AMP, T, C	bla_{CTX}
7	*S. Enteritidis*	E, P, AMX, S, NA, SXT, AMP, T	bla_{CTX}
8	*S. Enteritidis*	E, P, AMX, S, NA, SXT, AMP, T	bla_{CTX}, bla_{CMY}
9	*S. Enteritidis*	E, P, AMX, S, NA, SXT	bla_{CTX}, bla_{CMY}
10	*S. Enteritidis*	E, P, AMX, S, NA, SXT	bla_{CTX}
12	*S. Enteritidis*	E, P, AMX, S	bla_{CMY}
13	*S. Enteritidis*	E, P	bla_{CTX}
14	*S. Typhimurium*	E, P, AMX, S, NA, SXT, AMP, T, C, NOR, N, CIP, G, K	bla_{CTX}
15	*S. Typhimurium*	E, P, AMX, S, NA, SXT, AMP, T, C, NOR, N, CIP	bla_{CMY}
17	*S. Typhimurium*	E, P, AMX, S, NA, SXT, AMP, T, C, NOR	bla_{CTX}
18	*S. Typhimurium*	E, P, AMX, S, NA, SXT, AMP, T, C, NOR	bla_{CTX}, bla_{CMY}
19	*S. Typhimurium*	E, P, AMX, S, NA, SXT, AMP, T, C	bla_{CMY}
20	*S. Typhimurium*	E, P, AMX, S, NA, SXT, AMP, T, C	bla_{CTX}
22	*S. Kentucky*	E, P, AMX, S, NA, SXT, AMP, T, C, NOR, N, CIP	bla_{CMY}
24	*S. Kentucky*	E, P, AMX, S, NA, SXT, AMP	bla_{CTX}
26	*S. Kentucky*	E, P	bla_{CTX}, bla_{CMY}

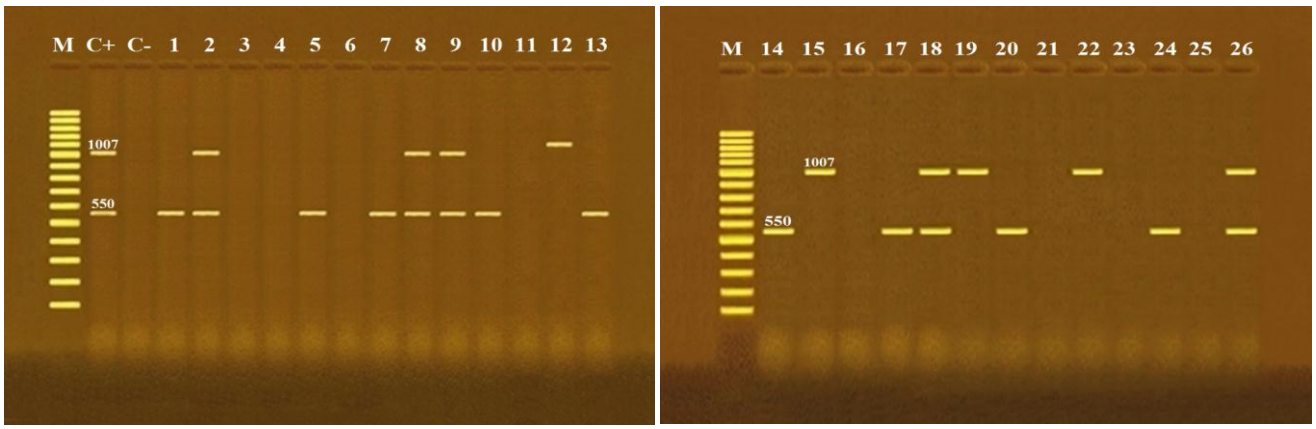

Fig. (1& 2): PCR amplification of the 550bp and 1007bp fragments of bla_{CTX} and bla_{CMY} genes, respectively, from 26 *Salmonella* isolates (1-26) showing positive amplicons migrates with the molecular DNA size marker (M)., C+ (control positive), C- (control negative). *S.* Enteritidis (1-13), *S.* Typhimurium (14-21), *S.* Kentucky (22-26)

1.5. Multiplex-PCR for detection of virulence associated genes (*stn, invA and hilA*).

Multiplex-PCR assay was done for 26 salmonella strains for detection of 3 virulence associated genes *stn, invA and hilA*. The results revealed that *invA* gene was found in all isolates (100%) while *stn* gene was found in 25/26 isolates (96.2%) arranged as follow; 12 *S. Enteritidis* (46.2%), 8 *S. Typhimurium* (30.8%) and 5 *S. Kentucky* (19.2%). Meanwhile, *hilA* gene was found in 22/26 isolates (84.6%) arranged as follow; 10 *S. Enteritidis* (38.5%), 8 *S. Typhimurium* (30.8%) and 4 *S. Kentucky* (15.4%) (Tables 3& 5 and Fig. 3& 4).

Table (5): Incidence of *stn*, *invA* and *hilA* virulence genes in isolated Salmonella.

Serotype (No.)	Virulence Genes					
	stn		*invA*		*hilA*	
	NO.	%	NO.	%	NO.	%
S. Enteritidis (13)	12	46.2	13	50	10	38.5
S. Typhimurium (8)	8	30.8	8	30.8	8	30.8
S. Kentucky (5)	5	19.2	5	19.2	4	15.4
Total (26)	25	96.2	26	100	22	84.6

%: calculated according to the Total No. of isolates (*n= 26*)

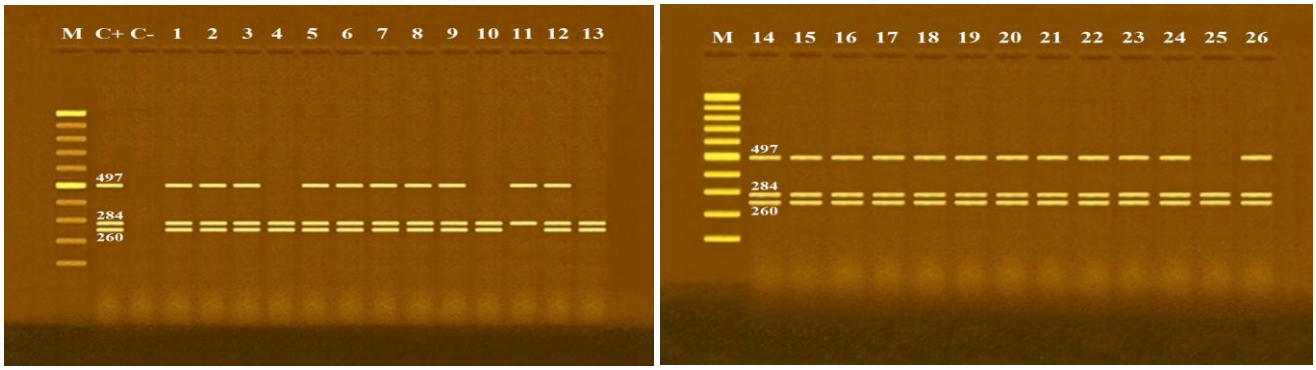

Fig. (3& 4): PCR amplification of the 260bp, 284 bp and 497bp fragments of *stn, invA* and *hilA* genes, respectively, from 26 *Salmonella* isolates (1-26) showing positive amplicons migrates with the molecular DNA size marker (M)., C+ (control positive), C- (control negative). *S.* Enteritidis (1-13), *S.* Typhimurium (14-21), *S.* Kentucky (22-26).

1. Discussion

Salmonellosis causes great mortalities and various morbidity changes as well as economic losses in poultry industry and a serious public health problem throughout the world **(Pedersen *et al.*, 2002)**. Identification and genotyping of *Salmonella* isolates are essential for epidemiological surveillance and investigations of outbreak.

In the current work, 26 *Salmonella* species were recovered from the 500 samples of internal organs (liver, heart, lung, airsacs, and kidney) of broiler chickens with prevalence rate of 5.2%. This result was similar to that obtained by reported by **Abd El-Galil *et al.* (1995)**; 6%, and nearly similar to those of **Abd El Fattah (2014)**; 8.72%, and **Radwan *et al.* (2016)**; 12%, while it was much lower than that reported by **Sharada *et al*. (1999)**; 30.5%.

Serotyping of 26 *Salmonella* isolates showed that *S.* Enteritidis, *S.* Typhimurium and *S.* Kentuky were identified at rates of 50%, 30.8% and 19.2%, respectively. The obtained results run parallel to that obtained by **Hegazy (2002)** who detected *S.* Enteritidis and *S.* Kentucky at rates of 62.16%, and 5.41% respectively.

Antimicrobial therapy is one of the primary control for reducing both the incidence and mortality associated with avian diseases therefore reducing their enormous losses in the poultry industry **(Radwan *et al.*, 2016)**. Increasing antimicrobial resistance is an important public health concern, and the emergence and spread of antimicrobial resistance is a complex problem driven by numerous interconnected factors. *In-vitro* antimicrobial susceptibility testing of veterinary pathogens can provide valuable guidance to the veterinarian in the choice of appropriate chemotherapy **(Radwan *et al.*, 2016)**. Moreover, it is very useful to detect the multidrug resistant isolates.

In the current study, the recovered *Salmonella* isolates (*n=26*) were subjected to *in-vitro* antimicrobial susceptibility tests against 14 different antimicrobial drugs to detect MDR isolates for further analyses of the isolates. Results illustrated in table (2) revealed that *Salmonella* isolates showed complete resistance against erythromycin and penicillin while they were highly resistant to amoxicillin and streptomycin (92.3% for both), nalidixic acid (80.8%), sulphamethoxazole-trimethoprime (76.9%), ampicillin (69.2%) and oxytetracycline (65.4%). On the other hand, they were highly sensitive to kanamycin (96.2%) then gentamycin (73.1%). MDR *Salmonella* isolates were 24 isolates with prevalence rate of 92.3%. Comparable results have been reported worldwide; in Egypt (**Ahmed *et al.*, 2009**), in USA (**Frye and Fedorka-Cray, 2007**), and in Italy (**Mammina *et al.*, 2002**), and in Portugal (**Antunes *et al.*, 2006**).

Beta-lactams belong to a family of antibiotics, the members of which have a β-lactam ring. Penicillins, cephalosporins, clavams (or oxapenams), cephamycins and carbapenems are members of this family. In Gram-negative bacteria, resistance to β-lactam antibiotics is primarily mediated by β-lactamases, which hydrolyze the β-lactam ring and thus inactivate the antibiotic. Many different β-lactamases have been described, but *TEM*-, *SHV*-, *OXA*-, *CMY*- and *CTX-M*-β-lactamases are the most predominant in Gram-negative bacteria. bla_{CTX-M} arise resistance to penicillins, extended-spectrum cephalosporins, and monobactams, and the enzymes are inhibited by clavulanate, sulbactam, and tazobactam. Typically, the CTX-M-ases hydrolyze cefotaxime more efficiently than ceftazidime, which is reflected in substantially higher MICs to cefotaxime than to ceftazidime (**Livermore and Woodford, 2006**).

In the present study 2 β-lactamase resistance genes (bla_{CTX} and bla_{CMY}) were evaluated in all salmonella isolates (*n=26*) using multiplex-PCR assay. The results illustrated in tables (3& 4) and Fig. (1& 2) revealed that bla_{CTX} gene was found in 14/26 isolates (53.9%) arranged as follow; 8 *S. Enteritidis* (30.8%), 4 *S. Typhimurium* (15.4%) and 2 *S. Kentucky* (7.7%). The resistance gene bla_{CTX} has previously been identified and reported increasingly in Gram-negative rods **(Bradford, 2001; Eckert, *et al.*, 2004; Naas, *et al.*, 2005 and Pitout, *et al.*, 2005)** and was characterized and isolated in Germany and Italy (**Barthelemy *et al.*, 1992 and Bauernfeind *et al.*, 1996)**. On the other hand, bla_{CMY} gene was found in 9/26 isolates (34.6%) arranged as follow; 4 *S. Enteritidis* (15.4%), 3 *S. Typhimurium* (11.5%) and 2 *S. Kentucky* (7.7%). The gene bla_{CMY} has previously been identified in *S.* Typhimurium and other serovars isolated from animals in Canada (**Allen and Poppe, 2002**), Egypt (**Ahmed *et al.*, 2009**) and USA **(Doublet *et al.*, 2004 and Frye and Fedorka-Cray, 2007**).

Salmonellae are invasive bacteria and harbor multiple systems for interacting with and penetrating the mucosal epithelium for systemic invasion (**Galan, 2001**). Indeed, a number of virulence-associated genetic regions, termed Salmonella pathogenicity islands (SPI), have been identified. The most two important SPI are SPI-1 and SPI-2 which encode the type three secretion systems, TTSS-1 and TTSS-2, respectively. These two SPI encode structural proteins that form needle-like complexes allowing the insertion of the bacterial proteins into the host cells that modulate the cellular functions and immune pathways (**Galan, 2001**). SPIs encode a number of virulence factors and play an important role in the pathogenicity of Salmonella. There are at least 60 genes associated with SPIs; many of these genes are encoded on (SPI-1); and the majority of them are located on the chromosome (*invA* and *phoP*) or on large virulence-associated plasmids (*spvC* and *spvA*). *Salmonella* strains lacking this SPI-1 have invasiveness rates towards Hela cells significantly lower than the rate of *invA*-positive strain STM1344 (**Li *et al,* 2011**). During the process of Salmonella infection, invasion genes are required for bacterial entry into host cells **(Groisman and Ochman, 1997)**.

Invasion-related gene *invA* has become one of the most popular PCR target sequences and its amplification now has been recognized as an international standard for detection of Salmonella and is important in its pathogenesis **(Radwan *et al.,* 2016)**. The *invA* gene encodes a protein in the inner membrane of bacteria which is responsible for invasion to the epithelial cells of the host **(Darwin and Miller, 1999)**. Also, *stn* virulence gene codes for enterotoxin production (**Murugkar *et al.,* 2003**). The *hilA* gene encodes an OmpR/ToxR family transcriptional regulator that activates the expression of invasion genes in response to both environmental and genetic regulatory factors (**Jennifer *et al,* 2003**).

In the present work, multiplex-PCR was applied on all salmonella isolates to determine 3 virulence-associated genes; *invA*, *stn* and *hila*. The results illustrated in tables (3& 5) and Fig. (3& 4) revealed that invA gene was detected in all isolates (100%) and *stn* gene was found in 96.2% of isolates while *hilA* gene was found in 84.6% of isolates. These findings supported that obtained by **Zou *et al.*** (**2012**) who found that the majority of the *Salmonella* isolates (99.3%) from human and food origin carried the *invA*, *stn* and other virulence genes.

Concerning *inv*A gene findings, the same results were obtained by other studies in Egypt **(Abd El-Ghany *et al.,* 2012 and Abd El Tawwab *et al.,* 2013**) as well as other studies around the world (**Amini *et al.,* 2010 and Campioni *et al.,* 2012**) where the *inv*A gene was present in all of the isolates from chickens. It was expected that this gene would be detected in all of the isolates due to its importance for cell invasion.

Regarding *stn* gene obtained results; comparable observations have been reported by other studies (**Murugkar *et al.,* 2003**, **Shome *et al.,* 2006 and Ezzat *et al.,* 2014**) where the *stn* gene was detected in all isolates (100%). Meanwhile the obtained data belonging *hilA* gene run parallel to other observations reported by other studies (**Campioni *et al.,* 2012 and Craciunas *et al.,* 2012**).

2. Conclusion

The presence of multidrug resistance pathogens occurred due to the misuse of the antibiotics and it is considered a great problem. In this work, multidrug-resistant strains of *S.* Enteritidis, *S.* Typhimurium and *S.* Kentucky from diseased broilers were recovered and identified. Furthermore, different resistance and virulence associated genes were analyzed using multiplex-PCR.

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