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Extended-spectrum β-lactamase-producing *Salmonella* serovars among healthy and diseased chickens and their public health implication

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ABSTRACT

Objectives: This study investigated the occurrence of extended-spectrum β-lactamase (ESBL)-producing *Salmonella* and the associated virulence genes among farmed chickens.
Methods: Cloacal swab samples were collected from apparently healthy and diseased chickens and were cultured for *Salmonella* using conventional methods. The isolates were serotyped using slide agglutination tests and were examined by polymerase chain reaction (PCR) for the virulence genes *invA*, *stn*, *svpC* and *pefA* and the outer membrane protein-encoding genes *ompA* and *ompF*. Screening for ESBL resistance was performed using the disk-diffusion test, the combinational-disk test with clavulanic acid, and multiplex PCR for bla_{TEM}, bla_{SHV}, bla_{CTX-M} and bla_{OXA}. The presence of the AmpC bla_{CMy-2} was tested among the ESBL-negative isolates by uniplex PCR. The resistant isolates were partially sequenced based on the *stn* gene.
Results: The *Salmonella* isolation rate was 3.4% (6/175) from healthy and 11.1% (14/126) from diseased chickens. The 20 isolates belong to serotypes with public health significance like Typhimurium, Kentucky and Infantis. All the isolates possess *invA*, *stn*, *svpC* and *ompF* genes; 16 isolates harboured *ompA*, and one carried *pefA*. Of the 20 isolates, 19 were resistant to more than one antibiotic. Of these 19 isolates, 16 were ESBL-producing with the majority carrying bla_{TEM} and bla_{SHV} genes. The four ESBL-negative isolates carried bla_{CMY-2}. Partial-*stn*-sequencing of the isolates revealed a high genetic relatedness to *Salmonella* strains from patients in Egypt and Asia.
Conclusions: Virulent ESBL-producing *Salmonella* was isolated from healthy and diseased chickens; the strains have a close relationship to human strains, posing a public health threat.

1. Introduction

Globally, *Salmonella* is considered as one of the most common etiological agents of foodborne diarrhoeal illnesses in humans [1]. Infection with *Salmonella* can be life threatening, depending on bacterial factors such as the serotype, virulence and susceptibility to antibiotic treatment [2]. The majority of *Salmonella* serotypes have a broad host range, they can be transmitted from animals to humans, causing gastroenteritis, which can be severe in children and immunocompromised patients [3]. Among these serotypes, Salmonella enterica serotypes Enteritidis, Typhimurium and Infantis predominate in most parts of the world [2]. A few serotypes are host-specific such as S. enterica serotypes Dublin, Cholerasuis and Gallinarum as well as the species *Salmonella* bongori, and infection of humans with these strains is often invasive and can be fatal [3]. The ability of *Salmonella* to cause disease is linked to a set of virulence genes, which are either located in chromosomes or carried on plasmids [4]. Chromosomal virulence genes encode for factors such as *invA* which allow *Salmonella* to invade epithelial cells, whereas the enterotoxin *stn* is involved in replication of *Salmonella* and has been shown to cause diarrhoea [5]. Moreover, the plasmid virulence genes are presumed to facilitate the systemic spread of *Salmonella* and colonisation of deep tissues [4] such as spv (*Salmonella* plasmid virulence) and pef (plasmid-encoded fimbriae).

The past few decades have shown the emergence of antibiotic-resistant *Salmonella*, particularly the extended spectrum β-lactamase (ESBL)-producing strains, which represents a significant challenge for human medicine [2]. The ESBL-producing bacteria

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carry a broad range of β-lactamase enzymes that hydrolyse a wide spectrum of penicillin and cephalosporin antibiotics, but not carbapenems [6,7]. Many β-lactamases are members of TEM (named after the patient Temoneira), sulfhydryl reagent variable (SHV), CTX-M (active on cefotaxime, first isolated at Munich), and OXA (oxacillinase) families [8,9]. The three enzymes TEM, SHV and CTX-M represent the most prevalent types of ESBL enzymes [7]. Recently, another group of β-lactamases named AmpC have been described, which confers resistance to all β-lactam antibiotics except, the fourth generation cephalosporins and carbapenems [10,11]. In contrast to ESBL, the AmpC group is not susceptible to β-lactam inhibitors, such as clavulanic acid, sulbactam and tazobactam [10]. The AmpC group can be encoded by genes located on chromosomes or plasmids [11]. In *Salmonella*, resistance to extended-spectrum cephalosporins were shown to commonly result from the action of the plasmid AmpC bla_{CMY-2} gene [12,13].

It has been reported that the outer membrane proteins (omp) of bacteria are involved in the antibiotic resistance. In this regard, the presence of the major ompA was linked to resistance of antibiotics in *E. coli* and *Acinetobacter baummani* [14]. However, the ompF porin, which allows the entry of antibiotics and other molecules to the bacterial cell, was downregulated in the resistant *E. coli* strains [15].

Globally, outbreaks of salmonellosis in humans have been linked to the consumption of contaminated foods of animal origin, particularly poultry and poultry products [1,2]. Chickens have been proposed to constitute a source for ESBL-producing *Enterobacteriaceae* that colonise and infect humans [16,17]. Therefore, the present study was carried out to (i) investigate the occurrence of ESBL-producing *Salmonella* spp. among farmed chickens; (ii) assess the presence of the virulence genes (*invA*, *stn*, *spvC* and *pefA*) and the outer membrane proteins (*ompA* and *ompF*); (iii) underline the public health importance of the current strains by analysing the genetic relation between the chicken-isolates and their relation to human strains from GenBank.

2. Methods

2.1. Collection of samples

Cloacal swabs were taken from chickens raised in farms located in Cairo, Giza, and the Al Qalyubia governorates, Egypt. Chickens included were: 175 apparently healthy (age mean = 89 days, age range: 20 days–1 year) and 126 diseased chickens having diarrhoea (age mean = 13.8 days, age range = 3–73 days). The samples were collected during the period from July 2017 to November 2018. The swab samples were pre-enriched in sterile buffered peptone water (Oxoid, Hampshire, UK) and were transported in an ice box.

Protocols for collection of samples and the used methods were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC; Number: VetCU0722019058) of the Faculty of Veterinary Medicine, Cairo University, Egypt.

2.2. Isolation and identification of Salmonella spp

This was performed according to the International Organization for Standardisation [18]. Briefly, the pre-enriched swab samples were incubated for 18 h at 37 °C. Then, 0.1 mL of the pre-enriched broth was transferred aseptically into 10 mL of Rappaport Vassiliadis (RV) enrichment broth (Oxoid) and incubated for 24 h at 42 °C. A loopful of the enriched RV broth was platted on Xylose Lysine Desoxycholate agar (XLD) (Oxoid) and incubated at 37 °C for 24–48 h. The suspected colonies were identified by Gram-stain films as well as biochemical identification using RapID ONE kit (Remel, USA).

2.3. Serotyping of the Salmonella isolates

Salmonella were serotyped using slide agglutination tests with known polyvalent somatic and flagellar antisera according to the Kauffmann–White serotyping scheme [19] in a reference laboratory for veterinary quality control on poultry production (Animal Health Research Institute, Dokki, Giza, Egypt).

2.4. Antibiotic susceptibility testing

Susceptibility of the *Salmonella* isolates to the following antibiotics were examined using the disk-diffusion test; cefpodoxime (CPD, 10 μg), cefotaxime (CTX, 30 μg), ceftazidime (CAZ, 30 μg), ceftriaxone (CRO, 30 μg) and aztreonam (ATM, 30 μg), (Oxoid). A confirmatory ESBL test was applied by using both cefotaxime and ceftazidime, alone and in combination with clavulanic acid (CTX 30 μg, CTX/clavulanic acid, 30/10 μg) and (CAZ 30 μg, CAZ/clavulanic acid, 30/10 μg). The tests were conducted, and the results were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute [20].

2.5. Molecular detection of ESBLs- and AmpC-(bla_{CMY-2}) encoding genes

DNA was extracted from the antibiotic-resistant isolates using the boiling method [21]. Multiplex polymerase chain reaction (PCR) was performed using specific oligonucleotide primers (Table 1) to detect the resistance determinant genes SHV, TEM, CTX-M and OXA [22]. PCR mixtures of 25 μL total volume, were prepared from 12.5 μL Emerald Amp GT PCR master mix (Takara Bio Inc., Shiga, Japan), 0.5 μL from each primer with concentration of 10 pmol, 3 μL template DNA from each isolate and 5.5 μL water. A negative control was included which contained all the components of the PCR mixture, except for the DNA which was replaced by water. All reactions were amplified using the following conditions: initial denaturation at 95 °C for 15 min; followed by 30 cycles of 94 °C for 30 s, 62 °C for 90 s and 72 °C for 60 s; then a final extension at 72 °C for 10 min. The PCR products were electrophoresed on 1.5% agarose gel, a 100-bp DNA ladder (size range: 100–1000 bp, Jenna Bioscience GmbH, Jenna, Germany) was run simultaneously to detect the size of the bands.

Isolates that were resistant to one or more of the examined antibiotics but were non-ESBLs-producing (n = 4) were subjected to uniplex PCR to determine the presence of the plasmid-mediated AmpC β-lactamase gene (bla_{CMY-2}) using specific oligonucleotide primers (Table 1) [23]. The PCR reaction mixtures of 25 μL total volume contain 12.5 μL of 2 × Emerald Amp GT PCR master mix (Takara Bio Inc), 8.5 μL water, 3 μL template DNA from each isolate, 0.5 μL from each primer with a concentration of 20 pmol. A negative control was included, which contained all the components of the PCR mixture, but with water instead of the template DNA. The PCR amplification was carried out with the following thermal profile; 94 °C for 5 min followed by 30 cycles of denaturation (94 °C for 1 min), annealing (61 °C for 1 min), extension (72 °C for 1 min), and a final extension at 72 °C for 5 min [23]. The PCR product was electrophoresed on a 1% agarose gel to determine the size of the product, a 100-bp DNA ladder (size range: 100–1000 bp, Jenna Bioscience GmbH, Jenna, Germany) was run simultaneously to detect the size of the bands.

2.6. Molecular detection of the virulence and outer membrane genes

The extracted DNAs from the antibiotic-resistant *Salmonella* isolates were also examined for the presence of the virulence genes *spvc*, *invA*, *stn* and the fimbriae *pefA*; the major outer membrane protein gene *ompA*, and the outer membrane porin gene *ompF*

Table 1
Sequence of oligonucleotide primers used for PCR amplification of β-lactamase and *Salmonella* virulence genes.

Genes [References]	Primer sequence (5′–3′)	Amplicon size (bp)	Company
bla_{TEM} [22]	CGC CGC ATA CAC TAT TCT CAG AAT GA ACG CTC ACC GGC TCC AGA TTT AT	445	Metabion, Planegg/ Steinkirchen Germany
bla_{SHV} [22]	CTT TAT CGG CCC TCA CTCAA AGG TGC TCA TCA TGG GAA AG	237	
bla_{CTXM} [22]	ATG TGC AGY ACC AGTAAR GTK ATG GC TGG GTR AAR TAR GTS ACC AGA AYC AGC GG	593	
bla_{OXA} [22]	ACA CAA TAC ATA TCA ACT TCG C AGT GTG TTT AGA ATG GTG ATC	813	
bla_{CMY-2} [23]	AGCGATCCGGTCACGAAATA CCCGTTTTATG CACCCATGA	695	
invA [24]	GTGAAATTATCGCCACGTT CGGGCAA TCATCGCACCGTCAAAGGAAGGAACC	284	
stn [25]	TTG TGT CGC TAT CAC TGG CAA CC ATT CGT AAC CCG CTC TCG TCC	617	
ompA [26]	AGT CGA GCT CAT GAA AAAGAC AGC TAT CGC AGT CAA GCT TTT AAG CCT GCG GCT GAG TTA	1052	
ompF [27]	CCTGGCAGCGGTGATCC TGGTGTAACCTACGCCATC	519	
pefA [25]	TGT TTC CGG GCT TGT GCT CAG GGC ATT TGC TGA TTC TTC C	700	
spvC [6]	ACCAGAGACATTGCCTTCC TTCTGATCGCCGCTATTCG	467	

PCR, polymerase chain reaction.

[6,24–27]. Uniplex PCR was carried out using specific oligonucleotide primers, displayed in Table 1. The PCR reaction mixtures of 25 μL total volume contain 12.5 μL of 2 × Emerald Amp GT PCR master mix (Takara Bio Inc), 4.5 μL water, 6 μL template DNA from each isolate, 1 μL from each primer with a concentration of 20 pmol. A negative control was included which contained all the components of the PCR mixture, but with water instead of the template DNA. The PCR reaction mixtures were amplified using the thermal profile conditions shown in Table 2. All PCR products were subjected to electrophoresis in a 1.5% agarose gel, the DNA ladder was run simultaneously. Two types of DNA ladder were used depending on the size of the PCR products, Gene ruler 100 bp (100–1000, Fermentas, Axon Scientific, Kuala Lampur, Malaysia); Gene pilot 100 bp plus (100–1500, Qiagen, MD, USA).

2.7. Sequencing and phylogenetic analysis of Salmonella enterotoxin stn gene

Amplicons of the *stn* gene from eight *Salmonella* strains were purified using a Qiaquick purification kit (Qiagen, Germany) and partially sequenced using a Big Dye Terminator V3.1 sequencing kit (Applied Biosystems). The obtained sequences were compared with those from the *stn* gene extracted from human isolates and retrieved from the Public GenBank Database using the National Center for Biotechnology Information-Basic Local Alignment Search Tool (NCBI-Blast) server. Multiple alignment was conducted using the BioEdit Clustal W program (version 7.0.1.4). A neighbour-joining-phylogenetic tree was built using MEGA7 software version 7.0.26. The eight *stn* sequences obtained were deposited in GenBank under the accession numbers MK695165 to MK695172.

3. Results

Salmonella was isolated from 6 (3.4%) of the 175 apparently healthy chickens examined (Table 3). Additionally, *Salmonella* was isolated from 14 (11.1%) of the 126 diseased chickens having diarrhoea that were examined. Serotyping of the 20 *Salmonella* isolates revealed a wide range of serovars (Table 3), of the six isolates from the apparently healthy chickens, three were *S.* Typhimurium, two were *S.* Warnow and one *S.* Giza. The 14 isolates from the diseased chickens include 3 *S.* Kentucky, 2 *S.* Tamale; whereas only 1 isolate was detected for each of the 9 following serovars; *S.* Giza, *S.* Infantis, *S.* Rechovot, *S.* Chemedy, *S.* Montevideo, *S.* Uno, *S.* Dalo, *S.* Senftenberg and *S.* Amsterdam. All the 20 isolates carried the chromosomal virulence genes *invA* and

Table 2
PCR amplification thermal conditions of the virulence genes.

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
invA	95 °C	94 °C	64 °C	72 °C	35	72 °C
	5 min	30 s	30 s	45 s		10 min
Stn	95 °C	94 °C	59 °C	72 °C	25	72 °C
	3 min	1 min	1 min	1 min		10 min
ompF	94 °C	94 °C	60 °C	72 °C	35	72 °C
	5 min	30 s	40 s	45 s		10 min
ompA	94 °C	94 °C	55 °C	72 °C	35	72 °C
	5 min	30 s	40 s	1 min		10 min
pefA	94 °C	94 °C	55 °C	72 °C	35	72 °C
	5 min	30 s	40 s	45 s		10 min
spvC	94 °C	94 °C	58 °C	72 °C	35	72 °C
	5 min	30 s	40 s	45 s		10 min

PCR, polymerase chain reaction.

Table 3
Occurrence of *Salmonella serovars* among apparently healthy and diseased chickens.

Chickens	Examined no.	Positive no. (%)	Serovars (no.)	Virulence genes
Healthy	175	6 (3.4%)	*S.* Typhimurium (3); *S.* Warnow (2); *S.* Giza (1)	The 20 isolates were positive to *invA, stn* and *svpc*
Diseased	126	14 (11%)	*S.* Kentucky (3); *S.* Tamale (2); *S.* Giza (1); *S.* Infantis (1); *S.* Rechovot (1); *S.* Chemedy (1); *S.* Montevideo (1); *S.* Uno (1); *S.* Dalo (1); *S.* Senftenbery (1); *S.* Amsterdam (1)	One isolate was positive to *pefA*
Total	301	20 (6.6%)		

stn, as well as the plasmid virulence gene *spvc*. The *pefA* gene was harboured only by one isolate of *S.* Typhimurium (Table 3).

Among the 20 *Salmonella* isolates, 20 were resistant to CPD, 18 to CTX, 16 to CAZ, 9 to CRO and 8 to ATM (Table 4**)**. The 19 suspected ESBL-producing strains were subjected to confirmatory test (CDT), which revealed that 16 isolates were ESBL-producing and 3 were non-ESBL-producing. The majority of the ESBL-producing strains (n = 9) possessed bla_{TEM} and bla_{SHV} genes, whereas, four isolates harboured bla_{TEM}, bla_{SHV} and $bla_{OXA;}$ one carried bla_{TEM}, bla_{SHV} and bla_{CTXM}; one carried bla_{TEM} and bla_{CTXM} and one carried bla_{SHV} and bla_{OXA}. The four non-ESBL-producing strains were further tested for the presence of the plasmid AmpC β-lactamase gene ($bla_{\text{CMY-2}}$) and were all positive. Moreover, we found that the isolate that was resistant only to CPD carried $bla_{\text{CMY-2}}$. Examination of the outer membrane proteins showed that all the isolates were *ompF* positive, whereas, the *ompA* gene was found in 16 isolates (Table 4).

Fig. 1 shows a high frequency (99%) of the current serotypes Warnow, Tamale, Giza, Rechovot and Kentucky to form a cluster (CI) together with an *S.* Arizona strain isolated from chickens in Egypt (KX079694.1). The CI cluster shared a common ancestor with cluster II (CII), which splits into two sub-clusters, CII.1 with *S.* Infantis and CII.2 including *S.* Typhimurium that grouped with *S.* Typhimurium chicken isolate from Korea (CP035301.1) and *S.* Typhimurium humans isolates from India (CP034968.1) and China (CP019442.1). A third cluster (CIII) was found which includes an *S.* Giza strain isolated in the current study, which showed a similar evolutionary pattern to an *S. enterica* strain isolated from a human in Egypt (LC227778.1) retrieved from GenBank.

4. Discussion

In the present study, we showed the isolation of *Salmonella* from apparently healthy chickens (3.4%, 6/175) and from diseased chickens with diarrhoea (11%, 14/126). All the 20 isolates were confirmed to be *Salmonella* by molecular detection of the *invA* gene, which is *Salmonella* genus-specific and used as a golden marker for diagnosis of *Salmonella* [24,28]. The isolated *Salmonella* were of the species *enterica* and showed a wide range of serotypes, encompassing Typhimurium, Kentucky, Warnow, Tamale, Giza, Infantis, Rechovot, Chemedey, Montevideo, Uno, Dalo, Senftenberg and Amsterdam. Our findings are slightly different from previous studies, that reported a prevalence of Typhimurium and Kentucky serotypes among *Salmonella* isolated from diseased chickens in Egypt [29–31]. Interestingly, the *Salmonella* enterotoxin *stn* gene was carried by the 20 isolates, even those from healthy chickens. This agrees with the findings that *stn* is involved in the regulation of bacterial cell membrane integrity [32]. Additionally, the *stn* gene was reported to be specifically distributed among *Salmonella enterica*, irrespective of their serotypes [32]. Of great concern is the presence of the *spvC* virulence gene in the current 20 isolates, which is consistent with other studies from Egypt (23%) and from Spain (73%) [33,34]. The *spv*C gene is located on a self-transmissible virulence plasmid, which facilitate the systemic spread of *Salmonella*, highlighting the potential high virulence of the current strains [35]. Furthermore, the *spvC* gene was shown to be present on IncF plasmids, that carry antibiotic-resistance genes [35]. As with the spvC gene, pefA is located on a virulence plasmid

Table 4
Antibiogram profile, β-lactamase encoding genes and *omp* genes of the isolated *Salmonella* serovars.

Isolates	Antibiogram						ESBLs-encoding genes				AmpC	Omp genes	
	CPD	CTX	CAZ	CRO	ATM	CDT	bla_{SHV}	bla_{TEM}	bla_{OXA}	bla_{CTXM}	$bla_{\text{CMY-2}}$	*ompF*	*ompA*
S. Typhimurium	R	R	S	I	S	**ESBL**	+	+	–	–	Nt	+	+
S. Typhimurium	R	I	I	S	S	**ESBL**	+	+	–	–	Nt	+	+
S. Typhimurium	R	R	R	S	S	**ESBL**	+	+	+	–	Nt	+	+
S. Warnow	R	I	S	S	S	**ESBL**	+	+	–	–	Nt	+	–
S. Warnow	R	R	R	R	I	**ESBL**	+	+	+	–	Nt	+	+
S. Giza	R	R	R	R	R	**ESBL**	+	+	+	–	Nt	+	+
S. Kentucky	R	I	I	R	S	non-ESBL	Nt				**+**	+	+
S. Kentucky	R	R	R	R	R	**ESBL**	+	+	–	–	Nt	+	+
S. Kentucky	I	S	I	S	S	non-ESBL	Nt				**+**	+	+
S. Tamale	R	R	R	R	I	**ESBL**	–	+	+	–	Nt	+	+
S. Tamale	R	S	S	S	S	Nt	Nt				**+**	+	+
S. Giza	R	R	R	S	R	**ESBL**	+	+	–	+	Nt	+	–
S. Infantis	R	R	I	S	S	**ESBL**	+	+	–	–	Nt	+	+
S. Rechovot	R	I	S	S	S	**ESBL**	+	+	+	–	Nt	+	–
S. Chemedey	R	I	I	S	R	**ESBL**	+	+	–	–	Nt	+	+
S. Montevideo	R	R	I	R	S	**ESBL**	+	+	–	–	Nt	+	+
S. Uno	R	R	R	R	R	**ESBL**	–	+	–	+	Nt	+	–
S. Dalo	R	R	R	R	S	non-ESBL	Nt				**+**	+	+
S. Senftenbery	R	R	R	S	R	**ESBL**	+	+	–	–	Nt	+	+
S. Amesterdam	R	R	R	S	S	**ESBL**	+	–	–	–	Nt	+	+

ATM, aztreonam; CAZ, ceftazidime; CDT, combinational disk test in the presence of clavulanic acid; CPD, cefpodoxime; CRO, ceftriaxone; CTX, cefotaxime; Nt, not tested.

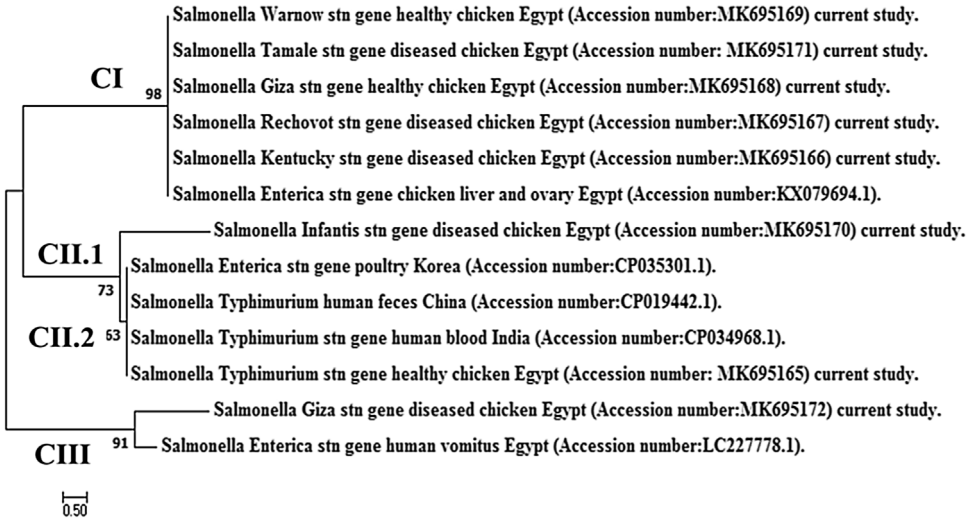

Fig. 1. Phylogenetic tree demonstrates the evolutionary history of the obtained *stn* gene sequences of *Salmonella* serovars from the current study and those of human cases retrieved from GenBank. The analysis was done using neighbour-joining method with MEGA7 software version 7.0.26.

[36]. In the present study, *pefA* was found in one isolate *of S.* Typhimurium, which agrees with Elkenany et al. [31]. Among the 20 isolates, 16 (80%) were ESBL-producing, which is higher than that reported in the USA (4.7%) and in the Netherlands (68%) [36,37]. Of particular public health importance is the fact that the six *Salmonella* strains isolated from heathy chickens were ESBL-producing, because such strains can easily spread within the chicken flocks and find its way to humans via contamination of poultry meat and eggs. Furthermore, the majority of the ESBL-producing strains carried the β-lactamase genes bla_{TEM} and bla_{SHV}, whereas the minority had bla_{OXA}, and only two isolates harboured bla_{CTXM}. This agrees with the resistance spectrum of TEM and SHV enzymes which extends to third generation cephalosporins and to aztreonam [7,8]. Previous studies in Egypt detected bla_{TEM} in resistant *Salmonella* isolates from chickens in a proportion of 96% and 100% [31,38]. In China, a study reported that the most common ESBL gene in *Salmonella*-resistant strains form poultry was bla_{CTXM}, followed by bla_{TEM}, bla_{OXA} and bla_{SHV} [39]. A recent study from Algeria showed a predominance of bla_{CTXM} over bla_{TEM}, whereas bla_{SHV} was not found [40]. This highlights a variation in the dominant type of ESBL-enzymes among regions and countries, which might be as a result of using different strategies for antibiotic treatment. Interestingly, our results demonstrated the presence of the AmpC bla_{CMY-2} gene among the resistant non-ESBLs-producing isolates. This agrees with the findings that the AmpC group is not susceptible to the β-lactam inhibitor clavulanic acid [10]. Additionally, previous studies demonstrated that bla_{CMY-2} mediates resistance to cephalosporins in *Salmonella* (14/16) isolated from retail chicken meat [12,13]. In Egypt, bla_{CMY-2}-carrying-*Enterobacteriaceae* other than *Salmonella* was isolated from retail chicken meat [11].

A major number of the current ESBL-resistant strains carried both *ompA* and *ompF* genes. Studies in *E. coli* suggested that the *ompF* facilitates the entrance of antibiotics to the bacterial cell [15]. However, the *ompA* maintains the cell membrane integrity and is linked to antibiotic-resistance [14]. Our findings indicate that beside the main role of ESBL genes, *ompA* might also contribute to the resistance-mechanism of *Salmonella* to ESBL. Bacteria that harbour ESBL-encoding genes can also carry genetic resistance determinants to other antibiotics, rendering infection with such bacteria serious because of the limited medication choices [35].

The current ESBL-producing isolates were partially sequenced, based on previous studies that reported that *stn* is widely distributed among *S. enterica*, irrespective of their serotypes or source (humans or animals) [3,4,32]. Indeed, we found a high genetic relatedness among the different isolates with close relatedness to *Salmonella* strains isolated from human patients in Egypt and Asia, indicating a significant public health concern.

In conclusion, we showed the presence of ESBL-producing *Salmonella* strains in diseased and healthy chickens, which are closely related to human *Salmonella* strains retrieved from the public database. The current isolates belong to serotypes known to be incriminated in human food outbreaks, like *S.* Typhimurium, *S.* Kentucky and *S.* Infantis. They predominantly carry the resistance determinant genes bla_{TEM} and bla_{SHV}, as well as the plasmid virulence *svpC* gene and the *ompA* gene, regardless of the state of the health the chickens. Previous studies reported that bla_{TEM} and *svpC* are carried on the same plasmid, which signifies the risk of spreading of virulent ESBL-strains. Furthermore, we reported the presence of the bla_{CMY-2} gene among the resistant non-ESBLs-producing isolates, indicating a role of the AmpC gene in mediating resistance to cephalosporins in *Salmonella.* This finding is worrisome, as the bla_{CMY-2} gene was shown to be carried on a large plasmid [10,13], facilitating its spread among different types of bacteria circulating in animals and humans.

Competing interests

The authors have no competing interests.

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Ethical approval

Not required. Protocols for collection of samples and the used methods were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC; Number: VetCU0722019058) of the Faculty of Veterinary Medicine, Cairo University, Egypt.

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