

RESEARCH ARTICLE

Antimicrobial Resistance, Virulence Associated Genes and Biofilm Formation of *Salmonella* Species Isolated from Different Sources

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Abstract

Infections with *Salmonella* species are among the major foodborne outbreaks of concern globally. This study aimed to characterize virulence determinants, antibiotic susceptibilities and biofilm formation ability of 29 *Salmonella* spp. isolated from chicken and human sources in Egypt. Molecular identification of virulence-associated genes revealed the detection of *avrA* gene in 100% of the examined isolates. The *sopB*, *stn*, *hilA* and *bcfC* genes were identified in 91.3% of the isolates, while, 86.2% and 31.03% harbored *mgtC* gene and *spvC* genes, respectively. The *pefA* and *fimH* genes were only identified in three isolates (10.3%). Fifteen antimicrobials were chosen to assess the susceptibility of the isolates to these drugs. The majority (31.03%) was resistant to 10 antibiotics; meanwhile, 89.6% were resistant to at least 5 antimicrobials. Out of 29 *Salmonella* isolates, 89.76%, 82.8% and 37.9% were biofilm producers at 35°C, 25°C, and 4°C, respectively. Significant correlation was observed between different *Salmonella* serotypes and their ability to produce biofilms. In conclusion, frequent monitoring of virulence determinants, antibiotic susceptibility and biofilm characteristics of *Salmonella* spp. is essential to improve food safety.

Keywords: *Salmonella* species, Virulotyping, Antibiotic Susceptibility, Biofilm Formation.

Introduction

Salmonella foodborne infection is of great impact in humans and animals worldwide [1]. Infection with *Salmonella* species has an estimated 1.3 billion incidences of nontyphoidal salmonellosis worldwide annually [2]. More than 2610 *Salmonella* serovars were recognized and almost all are able to cause illness in animals and humans [3]. *Salmonella enterica* serovar Typhimurium and *S. Enteritidis* are the most frequently serovars from human cases worldwide, with an overall proportion of 17.1 and 43.5%, respectively [4].

Salmonella spp. inhabit and colonize the intestinal tract of various animals including chickens, thus, they are considered the primary source of infection through consumption of contaminated meat, products and giblets

[5]. Studies estimated that 40% of the human clinical cases are attributed to the consumption of egg and poultry products [6, 7].

The pathogenicity of *Salmonella* spp. depends mainly on acquisition of virulence factors controlled by chromosomal or plasmid borne determinants. *Salmonella* pathogenicity island 1 (SPI1) is a region in the chromosomes that encodes for type III secretion system (TTSS) [8, 9]. The TTSS is essential for *Salmonella* virulence and regulated by *hilA* gene. Bacterial adherence to the intestinal epithelial cells is controlled by plasmid encoded fimbria locus associated gene (*pef*), while, cell invasion is encoded by the fimbrial *bcfC* gene [10, 11]. Moreover, bacterial invasion by deformation of membranes and host cell's cytoskeleton rearrangement is mediated by genes regulating *Salmonella* outer proteins

(*sop*) [12]. Cell apoptosis induction has a role in limiting the host's inflammatory response and this is mediated by the *avrA* gene [13]. Meanwhile, intracellular survival of *Salmonellae* is regulated by the *mgtC* genes and acute gastroenteritis is associated with enterotoxin production which is mediated by *stn* gene [14, 15]. The operon *spv*(RABCD) is located on plasmids and contains five genes, of which, *spvC* gene is essential for the survival of *Salmonellae* within the host macrophages [16].

Antibiotics are used in poultry sector for growth promotion, prophylaxis or therapeutic purposes. However, their indiscriminate usage resulted in the emergence of multiple drug resistance strains causing public health risk to consumers [17]. Resistant *Salmonella* spp. can be transmitted to humans through the food chain causing severe sequelae compared with infections caused by antimicrobial drug-susceptible species [18, 19].

Biofilms are exopolymer matrix of bacteria in different surfaces which allow the organisms to survive and persist in the environment and in the infected host [20]. The centers for diseases control and prevention (CDC) estimated that nearly 65% of all reported infections are caused by bacterial biofilms [21]. In environmental settings, *Salmonella* spp. is able to form biofilms on different biotic and abiotic surfaces that enable them to survive antibiotics and biocides, thus, enabling their transmission to other hosts [22, 23].

The study aimed to determine the antimicrobial resistance, virulence profiles and biofilm formation ability of *Salmonella* spp. isolated from chicken and human sources.

Material and methods

Salmonella isolates

Twenty-nine *Salmonella* isolates were recovered and confirmed by PCR and serotyping from chicken meat samples (n=9), giblets (n=4), egg shell (n=3), cloacal swabs (n=8) and surface swabs

from wooden cutting boards (n=2) at poultry pluck shop outlets in Sharkia Governorate, Egypt. In addition, two isolates from hand swabs of poultry workers at the outlets and one isolate from human stool samples at Outpatient Clinic were obtained [24]. The identified *Salmonella* serovars were *S. Typhimurium* (n=8), *S. Enteritidis* (n=7), *S. Newport* (n=6), *S. Kentucky* (n=7), and *S. Infantis* (n=3).

Molecular identification of virulence associated genes

The investigated virulence associated genes were *avrA*, *mgtC*, *sopB*, *stn*, *pefA*, *spvC*, *fimH*, *hilA*, and *bcfC*. The sequences of the primers and the sizes of the amplified products are listed in Supplementary Table (1). A positive control DNA from *S. Typhimurium* LT2 was used.

Antimicrobial resistance pattern of S. Typhimurium isolates

Antimicrobial susceptibility of *Salmonella* spp. was determined by the Kirby-Bauer method according to Clinical & Laboratory Standards Institute (CLSI). The used antibiotics were gentamicin (CN, 10 µg), streptomycin (S, 10 µg), amikacin (AK, 30 µg), kanamycin (K, 30 µg), nalidixic acid (NAL, 30 µg), ciprofloxacin (CIP, 5 µg), norfloxacin (NX, 10 µg), cefotaxime (CTX, 30 µg), ceftriaxone (CRO, 30 µg), chloramphenicol (CHL, 30 µg), Sulphamethazole/ Trimethoprim (SXT, 25 µg), erythromycin (E, 10 µg), tetracyclin (TE, 30 µg), colistin (CT, 10 µg) and ampicillin (AMP, 20 µg). Multiple antibiotic resistance (MAR) index was determined as the ratio of the number of the antibiotics to which *Salmonella* isolates displayed resistance to the number of drugs to which *Salmonella* isolates were examined [25]. Multidrug resistance (MDR) was defined as resistance of an isolate to at least one agent in three or more antibiotic classes [26]. *E. coli* ATCC 25922 was used as the quality control organism.

Biofilm formation ability

The ability of biofilm formation by *Salmonella* isolates was evaluated at 4°C, 25°C and 35°C using microplates of 96 wells as previously described [27]. The Optical Density (OD) of the stained adherent bacteria was determined with an ELISA reader (model: sunrise R4, serial no: 610000079) at wavelength of 620 nm (OD₆₂₀ nm) after the adjustment to zero of the negative control. The experiment was performed in triplicate and repeated three times, the data were then represented as mean and the standard deviation was calculated. The data obtained were used to classify the strains as non, weak, moderate and strong biofilm producers according to the following equations: Non-biofilm producer = $OD \leq OD_c$, Weak biofilm producer = $OD_c < OD \leq 2 \times OD_c$, Moderate biofilm producer = $2 \times OD_c < OD \leq 4 \times OD_c$, Strong biofilm producer, $4 \times OD_c < OD$ [28].

The production of biofilm is regulated by the *gcpA*, *csgD* and *adrA* genes. Identification of the three genes in the examined isolates was carried out. The sequences of the primers are illustrated in Supplementary Table (1).

The *csgD* gene was sequenced from two *Salmonella* spp. isolates classified phenotypically as non-producer and strong biofilm producer. The amplicon was extracted from the gel by QIAquick Gel Extraction Kits (Qiagen, S. A. Courtaboeuf, France) according to the manufacturer's guidelines. The purified products were sequenced with Big dye Terminator V3.1 Cycle Sequencing Kits (Perkin-Elmer, Foster city, CA) as described by the manufacturer. Sequence analysis was done by DNASTAR software (Lasergene version 7.2; DNASTAR, Madison, WI) and the two sequences were submitted to the GenBank which provided the two accession numbers of M+G242330 and MG242331.

Statistical analysis

Differences in biofilm formation degree at the three temperatures were examined by One-Way Analysis of variance (ANOVA) and LSD (Least Significant Difference). While, the differences in biofilm formation between the serotypes at the three temperatures were analyzed by Two-Way ANOVA. The test results were calculated by SPSS version 22 (IBM Corp. 2013, Armonk, NY). Data were presented as mean \pm SD and significance was considered at $P < 0.05$.

Results

Molecular identification of virulence associated genes

The molecular identification of virulence associated genes revealed the detection of *avrA* in all the examined isolates, while, *bcfC*, *sopB*, *stn* and *hilA* were identified in 93.1% of the isolates, each. The *mgtC*, *spvC*, *pefA* and *fimH* were detected in 82.8%, 31.03%, 10.3% and 10.3% of salmonella isolates, each.

Antimicrobial resistance

Fifteen antimicrobials were chosen during the current study to assess the susceptibility of the isolated *Salmonella* spp. strains (n=29) to these drugs (Table 1). All the examined isolates showed resistance against erythromycin, while, 96.5% were resistant to cefotaxime, nalidixic acid and colistin, each.

All the examined *Salmonella* spp. isolates were multidrug resistant (MDR) showing resistance to at least three drugs, the (31.03%) were resistant to 10 antibiotics, meanwhile, 89.6% of the isolates were resistant to at least 5 antimicrobials. The average MAR index was 0.53, 69% of the isolates had MAR index above the average.

Table 1: Antimicrobial susceptibility of *Salmonella* isolates to different antibiotics

Antimicrobials (abbreviation)	<i>Salmonella</i> isolates (no = 29)		
	*R	*I	*S
Gentamicin (CN)	19 (65.5%)	-	10 (34.5%)
Streptomycin (S)	24 (82.7%)	3 (10.3%)	2 (7%)
Amikacin (AK)	-	3 (10.3%)	26 (89.7%)
Tetracyclines (TE)	26(89.7%)	-	3(10.3%)
Kanamycin (K)	21(72.5%)	8(27.5%)	-
Nalidixic acid (NA)	28 (96.5%)	-	1 (3.5%)
Ciprofloxacin (CIP)	7 (24%)	16 (55.3%)	6(20.7%)
Norfloxacin (NOR)	7 (24%)	1 (3.5%)	21 (72.5%)
Cefotaxime (CTX)	28 (96.5%)	1 (3.5%)	-
Colistin (CT)	28(96.5%)	1 (3.5%)	-
Sulphamethazole/ Trimethoprim (SXT)	23 (79.3%)	-	6 (20.7%)
Erythromycin (E)	29 (100%)	-	-
Ampicillin (AMP)	16 (55.2%)	-	13 (44.8%)
Chloramphenicol (CHL)	22 (75.7%)	-	7 (24.3%)
Ceftriaxone (CRO)	2 (7%)	5 (17.3%)	22 (75.7%)

*R: resistant, I: intermediate, S: sensitive

Biofilm formation

Out of 29 *Salmonella* isolates, 26 (89.76%) were biofilm producers, where, 9 (31.03%), 11 (37.9%) and 6 (20.7%) were classified as weak, moderate and strong biofilm producers, respectively, at 35°C (Table 2). At 25°C, 24 (82.8%) were biofilm producers, of which, 7 (24.1%), 11 (37.9%) and 6 (20.7%) were weak, moderate and strong producers, respectively. However, at 4°C, only 11 (37.9%) produced biofilm, where, 4 (13.8%) and 7 (24.1%) were weak

and moderate biofilm producers, respectively. Overall, there was a significant effect of temperature on the ability of *Salmonella* isolates to produce biofilm ($p \leq 0.001$). A significant correlation was observed between different *Salmonella* serotypes and their ability to produce biofilms ($p \leq 0.001$) (Figure 1). The results in the current study showed the distribution of *adrA* and *csgD* in 100% of the isolates, while *gcpA* gene was identified in 28 (96.6%) of the isolates.

Table 2: Biofilm formation in *Salmonella* species at 4°C, 25°C and 35°C

Temperature	Non-producer	Degree of biofilm production (%), Average OD±SD			Overall biofilm producers
		Weak	Moderate	Strong	
4°C	18 (62.1, 0.0684±0.046)	4 (13.8, 0.1560±0.048)	7 (24.1, 0.3657±0.049)	-	11 (37.9%)
25°C	5 (17.3, 0.059±0.029)	7 (24.1, 0.1630±0.034)	11 (37.9, 0.3376±0.067)	6 (20.7, 0.7883±0.052)	24 (82.8%)
35°C	3 (10.3, 0.0294±0.021)	9 (31.03, 0.1647±0.039)	11 (37.9, 0.3494±0.068)	6 (20.7, 0.7923±0.058)	26 (89.7%)

OD: Optical Density

SD: Standard Deviation

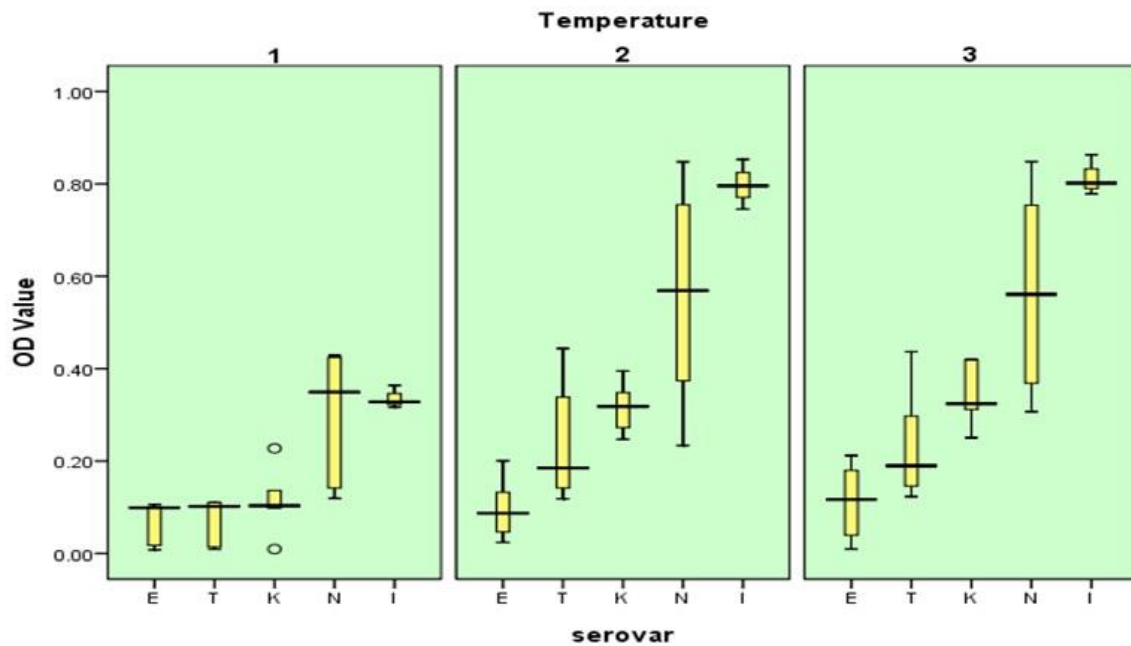


Figure 1: Boxplot showing the median optical density of biofilm formation by different *Salmonella* serovars at different temperatures (1: 4°C, 2: 25°C and 35°C), E: *S. Enteritidis*, T: *S. Typhimurium*, K: *S. Kentucky*, N: *S. Newport* I: *S. Infantis*.

Discussion

Salmonella spp. is the most notified foodborne pathogen with 70% of infections related to poultry as estimated by Rapid Alert System for Food and Feed annual report [29]. Chicken meat and products were associated with increased incidence of salmonellosis in many developing countries including India, Egypt, Brazil and Zimbabwe [30].

Virulotyping is an approach to understand how various virulence genes reflect bacterial properties [31]. Twenty-nine *Salmonella* isolates representing five different serovars (*S. Typhimurium*, *S. Enteritidis*, *S. Newport*, *S. Kentucky* and *S. Infantis*) were subjected to PCR for the detection of some encoded virulence determinants.

The detection of *avrA* gene in all the investigated isolates was in agreement with other studies [11, 32]. The high frequency of this gene is only observed in serovars that have a potential to cause severe salmonellosis in humans [11]. Lower

frequencies of 80% [33] and 50% [34] were also documented. This variation could be attributed to recombination which frequently occurs in the location of this gene [35].

The detection of *stn* gene in 91.3% of *Salmonella* isolates was comparable with other studies in Egypt [32, 36]. This gene was previously reported to be widely distributed among different *Salmonella* serovars [15]. However, lower rates were reported in the same study area; 11.1% [37] and 58.8% [38].

Consistent with our results, the *hilA* gene was previously identified in 100 and 88.2% of *Salmonella* spp. isolated from chicken samples in Brazil [11] and Egypt [38], respectively. While the gene was identified in 8.3% [37] and 8.6% [39] in other studies.

Salmonella spp. outer proteins (Sops) deform the membranes and rearrange the cytoskeleton of host cells to facilitate the invasion of this organism [12, 40]. Nearly Similar results were reported in USA [41,

42] and Egypt [32]. Comparable detection rates of the *spvC* gene in *Salmonella* isolates were reported in Egypt [36, 38]. Meanwhile, higher percentages were recorded in different studies worldwide [11, 43, 44].

The *bcfC* gene is widely distributed among isolates from animals, humans and environment, but with some diversity [4] which could be explained by serovar specificity of virulence plasmids [45]. The *mgtC* gene encodes a membrane protein that affects host-pathogen interactions, either by slowing the apoptotic process or by protecting the bacterium from host cell defenses [46]. The *bcfC* and *mgtC* genes were also reported in isolates from chicken and human sources in Egypt [32] and Europe [31].

Fimbriae play an important role in the pathogenicity of *Salmonella* spp. because they promote their attachment to epithelial cells. The *pefA* gene was reported to be serovar specific in only *S. Typhimurium* and *S. Enteritidis* [47, 48]. This gene was identified in only three *S. Typhimurium* isolates (10.3%) in our study. The obtained low frequency of *pefA* gene was comparable with other findings [17, 32].

The considerable differences in virulence determinants of *Salmonella* serovars are attributed to the variation in sample sources, types of serovars and presence or absence of plasmids carrying these genes [49].

The development of antibiotic resistant bacteria is resulted from the uncontrolled use of antimicrobials in food animals and transmission of resistance to human isolates through the food chain [50]. Ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole were the drugs of choice for *Salmonella* spp. infection treatment for decades [51]. However, due to the increased resistance to these drugs, fluoroquinolones (in adults) and extended spectrum cephalosporins (in children) have become common for treatment [52, 53]. In some cases of resistance to both fluoroquinolones

and cephalosporins, salmonellosis treatment is problematic [54].

The results revealed that all the isolates exhibited resistance against erythromycin, indicating its limited therapeutic value and this is comparable with other studies [36, 37, 55]. Of the examined isolates, 96.5% were resistant to cefotaxime and nalidixic acid. Comparable results ranging from 92.8%-100% resistance to nalidixic acid were reported [36, 37, 56, 57]. In contrary, other studies reported high sensitivity of *Salmonella* spp. to cefotaxime [32, 57, 58].

Due to the recent emergence of MDR gram negative bacteria, case reports have shown that colistin can be used in treatment with minimal adverse outcomes [59, 60]. Consequently, resistant *Salmonella* isolates have emerged due to the decreased binding affinity of colistin to *Salmonella* lipopolysaccharides [61]. Our results clarified high resistance of *Salmonella* isolates to colistin (96.5%). This was in agreement with other results [55, 56]. In contrary, no *Salmonella* isolates of chicken origin showed resistance to colistin in Iran, due to the limited availability and the high cost of the drug [57].

Fortunately, 75.7% of the isolates, including all *S. Typhimurium* and *S. Enteritidis* serovars were sensitive to ceftriaxone. This relatively low resistance is of great concern because this drug is used for the treatment of salmonellosis in children [32, 62]. On the contrary, 50% of *S. Typhimurium* isolates from diarrheic patients were resistant to the drug [37].

The high susceptibility of *Salmonella* isolates to amikacin, ciprofloxacin and norfloxacin in this study could be due to the limited use of these drugs because they are expensive and not being sold in private pharmacies without prescriptions.

Worldwide concerns are increased due to the emergence of multidrug resistant pathogens including *Salmonella* spp. [63]. All the examined isolates were resistant to at least three drugs. The majority of the

isolates (31.03%) were resistant to 10 antibiotics; meanwhile, 89.6% were resistant to at least 5 antimicrobials. Several studies reported also high percentages of MDR *Salmonella* isolates to at least three antibiotics [32, 36, 38, 48, 64].

The emergence of multiple drug resistance requires strict measures against the uncontrolled use of antibiotics by farmers, veterinarians and physicians [64]. Exposure of an organism to one antibiotic may result in resistance to any other drug without previous exposure due to co-selection of resistance determinants [65, 66]. In the present study, significant correlation was observed between nalidixic acid resistance and *bcfC*, *mgtC*, *sopB*, *stn* and *fimH* genes. Moreover, an association between gentamicin resistance and *bcfC*, *hilA* and *sopB* genes was also clear. In accordance, El-Atfehly [67] reported an association between resistance of *Salmonella* spp. to the commonly used antibiotics and different virulence associated genes in Egypt.

Increased virulence may evolve due to increased resistance to antibiotics, thus, control of resistance spread is required together with virulence spread control [68]. This can be achieved by understanding the regulation of virulence and antibiotic resistance.

It has been shown that MAR higher than 0.2 could be due to contamination from high risk sources such as humans and farm animals frequently exposed to antibiotics, thus resulting in potential risk to consumers [25]. Out of the isolates, 69% had MAR index above the average of 0.53, this is consistent with another study in Egypt [36]. The high level of antibiotic resistance exhibited by *Salmonella* spp. in this study suggests more restrictions on the irrational use of antibiotics.

The formation of biofilms on polystyrene is of concern because this material is used in food industries and kitchens [23]. Inefficient cleaning of the cutting surfaces in pluck shops or kitchens

can result in formation of biofilms on these surfaces, thus, enhancing bacterial tolerance to disinfectants and promoting cross contamination [69]. Out of 29 *Salmonella* isolates, 89.76%, 82.8% and 37.9% were biofilm producers at 35°C, 25°C and 4°C, respectively. Overall, there was a significant effect of temperature on the ability of *Salmonella* isolates to produce biofilm ($p \leq 0.001$). In accordance, 85% *Salmonella* isolates were biofilm producers on polystyrene microtiter plates at 37°C, of which, 67.5% and 17.5% were weak and moderate producers [70]. In Spain, out of 61 *Salmonella* isolates from poultry, 57.4% were classified as weak, 36.1% moderate and 19.8% strong biofilm producers after overnight incubation at 37°C [71]. Out of 30 *Salmonella* strains, 97% produced biofilm at 30°C, while, 93% and 90% produced biofilm matrix at 37°C and 22°C, respectively [72]. Another study reported the ability of *S. Typhimurium* isolates from different species of birds in Iran to produce biofilms [73]. The observed differences in biofilm formation between the aforementioned studies could be attributed to several factors including strain variation, incubation time, media and temperature [70, 74].

The percentage of weak biofilm producers cannot be under estimated especially in case of *Salmonella* isolates originated from poultry and environmental sources because they could be exposed to external stress or over use of antibiotics and disinfectants resulting in acquisition of virulence potential [70, 75].

The influence of temperature is clear by increasing the incubation temperature compared to refrigeration at 4°C. The number of non-biofilm producers was significantly higher at 4°C than at 25°C and then at 35°C ($p \leq 0.05$). Strong biofilm producers were only observed at 25°C and 35°C. Regardless the species of microbes or the examined surface type, maximum adhesion intensity is observed when bacteria are kept at temperatures next to their optimum growth temperature [76].

These findings highlight the importance of raw poultry storage at refrigeration temperature.

A significant correlation was observed between different *Salmonella* serotypes and their ability to produce biofilms ($p \leq 0.001$). Likewise, considerable differences in biofilm formation among *Salmonella* serovars were observed [71, 77]. In contrary, no difference between *Salmonella* serovars of variable origin and their ability for biofilm production were reported [70, 78].

The distribution of *adrA* and *csgD* genes was 100% in the examined isolates, while *gcpA* gene was identified in 96.6% of the isolates. Although *adrA* and *csgD* genes were identified in all isolates; 62.1%, 17.3% and 10.3% were classified as non-producers at 4°C, 25°C and 35°C, respectively. This indicates that other genes may be involved in this process. Similar findings were also reported in several studies [69, 77, 79].

Sequencing of the fimbriae regulator gene (*csgD*) was performed from two *S. Typhimurium* isolates, the first one was classified as non-biofilm producer and the other one was strong producer at 35°C. The results revealed that the sequences of the two isolates shared 100% identity with each other and with other *Salmonella* isolates on the GenBank. These findings indicated that the gene is detected in both *S. Typhimurium* isolates phenotypically classified as non-producer or strong biofilm producers. Therefore, it is not possible to state that a strain is biofilm producer based only on the molecular amplification of *csgD* and *adrA* genes [80].

=Conclusion

The present study demonstrated the presence of potentially pathogenic *Salmonella* species in chicken and human sources in Egypt. These findings provide important insights onto hygienic measures that should be applied in pluck shop markets. The high MAR index of the isolates and their ability to form biofilm at

high temperatures constitute public health hazards.

Conflict of interest

The authors declare that there are no competing interests.

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الملخص العربي

مقاومة المضادات الحيوية والجينات المرتبطة بالضراوة وتكوين الشريط الحيوي لأنواع السالمونيلا المستفردة من مصادر مختلفة

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تعد العدوى بأنواع السالمونيلا من أهم الاندلاعات الكبرى للعدوى بالغذاء وذات اهتمام عالمي. استهدفت الدراسة الحالية توصيف محددات الضراوة والحساسية للمضادات الحيوية والقدرة على تكوين الشريط الحيوي لعدد 29 مستفردة من نوع السالمونيلا من أصل داجني وأدمي بمصر. أسفر التعريف الجزيئي لجينات مرتبطة بالضراوة عن تحديد جين *avrA* في كل المستفردات التي فحصت. بينما عرفت جينات *sopB*, *stn*, *hila* and *bcfC* بنسبة 91.3% من المستفردات. بينما وجد أن 86.2% و 31.03% تحتوي على جين *mgtC* و جين *spvC*، على التوالي. كما وجد جيني *fimH* و *pefA* بعدد 3 (10.3%) مستفردات فقط. تم اختيار عدد 15 مضاد حيوي لتقييم حساسية المستفردات لها. اتضح أن 31.03% من المستفردات محل الدراسة مقاومة لعدد 10 مضادات حيوية، بينما 89.6% كان مقاوماً على الأقل لعدد 5 مضادات حيوية. وباختبار عدد 29 مستفردة سالمونيلا، وجد أن 89.76%، 82.8% و 37.9% منتجاً للشريط الحيوي عند درجة حرارة 35، 25 و 4 درجة مئوية، على التوالي. لوحظ ارتباطاً معنوياً بين أنواع السالمونيلا المصلية وقدرتها على انتاج الشريط الحيوي. وتم استنتاج أن المتابعة المتكررة لمحددات الضراوة والحساسية للمضادات الحيوية وانتاجية الشريط الحيوي لأنواع السالمونيلا تعد من الضروريات لتحسين سلامة الغذاء.