Prevalence, Virulence and Antimicrobial Resistance of *Salmonella* Typhimurium in Retail Chicken Meat

Marwa El Sayed Abo Hashem; Ezzat, Mahmoud; Bolbol, Mohamed; Reham M. El-Tarabili

Bacteriology, Immunology and Mycology Department, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.

Abstract

Salmonella Typhimurium was isolated from food-borne outbreaks basically. The study pointed to reveal the overall prevalence of *Salmonella* in retail chicken meat, serotyping of *Salmonella* isolates to detect *Salmonella* Typhimurium prevalence in retail chicken meat, antimicrobial susceptibility testing of recovered *Salmonella* Typhimurium isolates, and *stn*, *hil*A, and *pef*A as virulence genes and bla_{TEM}, bla_{CTX-M}, bla_{SHV}, bla_{CMY}, bla_{DHA}, *qnr*A, *aad*A1, *tet*A and *sul*1 as antimicrobial resistance genes. Out of 75 chicken meat samples, Two samples were positively identified as *Salmonella* with a total prevalence of 2.7 % (2/75). *Salmonella* isolates were serologically identified as *Salmonella enterica* serovar *Typhimurium*. All confirmed *Salmonella* isolates showed resistance to tetracycline (tetracycline), penicillins (ampicillin), aminoglycoside (gentamycin and streptomycin), sulphonamide (trimethoprim /sulfamethoxazole) and cephalosporins (cefuroxime). In comparison, 50% of the isolates were resistant to cephalosporins (cefotaxime and ceftazidime), nitrofuran (nitrofurantoin), and fluoroquinolones (levofloxacin and ciprofloxacin). Phenotypically, 50% of recovered isolates showed XRD (extensively drug resistance) to eight classes and 50% showed MRD (multidrug resistance). Concerning *pef*A, *stn*, and *hil*A as virulence genes, *stn* and *hil*A were detected in all tested *Salmonella* Typhimurium by 100% (2/2), while *pef*A was not detected (0/2). Furthermore, all the following antimicrobial resistance genes were detected by 100 % as bla_{TEM}, bla_{CTX-M} and bla_{SHV}, bla_{CMY}, *aad*A1, *tet*A, and *sul*1. While bla_{DHA} was not detected but *qnr*A was detected by 50%. In conclusion, raw retail chicken meat is heavily contaminated with XDR *Salmonella* Typhimurium. Moreover, important virulence factors in the isolates were also present, expanding the scope for public health concerns due to the potential for cross-contamination with other foods and the eating of undercooked poultry.

Keywords: *Salmonella* Typhimurium, serotyping, antimicrobial susceptibility testing, virulence genes, antimicrobial resistance genes.

Introduction

Poultry meat has long been regarded as a nutrient-dense food source because of its high protein, low fat, and beneficial unsaturated fatty acid concentration ***(Mulder, 1999).*** *Salmonella* outbreaks have been linked to the ingestion of various food items, primarily those derived from animals ***(Hernandez et al., 2005).*** Probably, poultry products could be contaminated with *Salmonella* during the multiple steps of food production: processing, distribution, retail marketing, handling, and preparation ***(Dookeran et al., 2012).***

Salmonella Typhimurium is a gram-negative bacillus, aerobic to facultatively anaerobic, motile and non-spore-forming bacteria ***(Delano et al., 2002).*** For information, the standard conventional cultural methods used in *Salmonella* spp. isolation and identification are time-consuming; it may need 7 days to confirm *Salmonella* spp. identification. Hence, simple, rapid, sensitive, and selective *Salmonella* detection was achievable through polymerase chain reaction (PCR) based methods ***(Malorny et al., 2003).*** Multidrug resistance has been assessed as an emerging and serious problem worldwide in the last decade and has a critical public health threat. The emergence of XDR and MDR bacterial pathogens from different origins, including poultry, fish, animals, food products, and humans, was reported recently in several investigations ***(Algammal et al., 2021a, Algammal et al., 2021b, Hetta et al., 2021).***

Materials and methods

1. Sample collection

From several marketplaces in Cairo, 75 samples of chicken flesh were randomly selected for testing. Samples were immediately transported to the laboratory in an ice box, where they were kept frozen until further analysis was completed. Incubation in the refrigerator overnight resulted in thawing ***(Roberts and Greenwood 2003).***

2. Isolation and identification of *Salmonella*

To isolate *Salmonella*, samples were pre-enriched in Rapport–Vassiliadis broth (Oxoid, Hampshire, UK), then incubated at 37°C for 18-24 hours before being selectively cultured on Salmonella–Shigella (SS) agar (Oxoid, Hampshire, UK) and xylose lysine deoxycholate (XLD) agar (Oxoid, Hampshire, UK). The putative colonies were recognized based on colonial characteristics, Gram staining microscopical analysis, and biochemical reactions (oxidase test, catalase test, indole, methyl-red, citrate-utilization, H2S, urease, and Voges-Proskauer).

3.Serotyping of *Salmonella*

Salmonella isolates were serotyped using *Salmonella* antiserum according to the Kauffman – White scheme ***Kauffman (1974)*** for the identification of Somatic (O) and flagellar (H) antigens

4. Antimicrobial susceptibility of *Salmonella*

Isolates were tested against 11 antibacterial agents (Oxoid Hampshire, UK), including ampicillin, cefuroxime, cefotaxime, ceftazidime, gentamycin, streptomycin, ciprofloxacin, levofloxacin, nitrofurantoin, tetracycline, and trimethoprim/sulfamethoxazole.

Antibiogram was performed using broth microdilution method as described by ***Wang and Gu (2021),*** and results were interpreted according to ***CLSI (2018).***

5. Molecular detection of some virulence and antimicrobial resistance genes *Salmonella* isolates

PCR was used for the detection of *stn* (*Salmonella* enterotoxin), *hil*A (gene encodes an ompR/ToxR family transcriptional regulator) and *pef*A(plasmid encoded fimbriae) as virulence genes as shown in Table (1) and ESBL (bla_{TEM}, bla_{CTX-M} and bla_{SHV}), ampC genes (bla_{CMY} and bla_{DHA}), quinolones (*qnr*A), aminoglycoside (*aad*A1), tetracycline (*tet*A) and sulphonamide(*sul*1) as antimicrobial resistance genes as shown in Table (2). QIAamp DNA Mini kit (Qiagen, GmbH, Germany/Catalogue No.51304) was used for DNA extraction according to the manufacturer's recommendations. DNA amplification of *Salmonella* virulence and antimicrobial resistancegenes were performed according to references shown in Tables (1,2).

Table 1. *Target genes, primer sequences, specific amplicon size of Salmonella virulence genes*

Bacteria	Gene	Sequence	Amplified product	Reference
Salmonella	***pef*A-F**	**TGTTTCCGGGCTTGTGCT**	**700 bp**	**Murugkar *et al.*, 2003**
	***pef*A-R**	**CAGGGCATTTGCTGATTCTTCC**		
	***stn*-F**	**TTGTGTCGCTATCACTGGCAACC**	**617 bp**	
	***stn*-R**	**ATTCGTAACCCGCTCTCGTCC**		
	***hil*A-F**	**CGGAAGCTTATTTGCGCCATGCTGAGGTAG**	**854 bp**	
	***hil*A-R**	**GCATGGATCCCCGCCGGCAGAGTTGTG**		

Table 2. *Target genes, primer sequences, specific amplicon size of Salmonella antimicrobial resistance genes*

Gene family	Gene	Sequence	Amplified product	Reference
ESBL	*bla*$_{TEM}$	ATCAGCAATAAACCAGC	516 bp	Colom et al., 2003
		CCCCGAAGAACGTTTTC		
	bla$_{CTX-M}$	ATG TGC AGY ACC AGT AAR GTK ATG GC	593 bp	
		TGG GTR AAR TAR GTS ACC AGA AYC AGC GG		
	bla$_{SHV}$	AGGATTGACTGCCTTTTTG	392 bp	
		ATTTGCTGATTTCGCTCG		
AmpC	*bla*$_{CMY}$	GCTGCTCAAGGAGCACAGGAT	520 bp	Schill et al., 2017
		CACATTGACATAGGTGTGGTG		
	bla$_{DHA}$	AACTTTCACAGGTGTGCTGGGT	405 bp	
		CCGTACGCATACTGGCTTTGC		
Quinolones	*qnr*A	ATTTCTCACGCCAGGATTTG	516 bp	Robicsek et al., 2006
		GATCGGCAAAGGTTAGGTCA		
Aminoglycoside	*aad*A1	TATCAGAGGTAGTTGGCGTCAT	484 bp	Randall et al. 2004
		GTTCCATAGCGTTAAGGTTTCATT		
Tetracycline	*tet*A	GGTTCACTCGAACGACGTCA	576 bp	
		CTGTCCGACAAGTTGCATGA		
Sulfonamide	*sul*1	CGGCGTGGGCTACCTGAACG	433 bp	Ibekwe et al., 2011
		GCCGATCGCGTGAAGTTCCG		

Results

1. Prevalence of *Salmonella* in retail chicken meat

Microscopically, *Salmonella* exhibited as Gram-negative medium-sized motile non-sporulated rods. *Salmonella* exhibited red colonies with a black center on XLD agar but as colorless colonies with a black center on SS agar. Catalase, methyl-red, citrate utilization, and H2S generation tests were positive in all isolates and negative for oxidase, indole, Voges-Proskauer, and urease.*Salmonella* was positively found in 2 of 75 chicken meat samples, for a total incidence of 2.7%.

2. Serotyping of *Salmonella* isolates

Salmonella isolates were serotyped as *Salmonella enterica* serovar Typhimurium.

3 Antimicrobial susceptibility testing of *Salmonella* Typhimurium

All confirmed *Salmonella* isolates showed resistance to tetracycline (tetracycline), penicillins (ampicillin), aminoglycoside (gentamycin and streptomycin), sulphonamide (trimethoprim

/sulfamethoxazole) and cephalosporins (cefuroxime). In comparison, 50% of the isolates were resistant to cephalosporins (cefotaxime and ceftazidime), nitrofuran (nitrofurantoin), and fluoroquinolones (levofloxacin and ciprofloxacin) (Figure 1). Phenotypically, 50% of recovered isolates showed XRD (extensively drug resistance) to eight classes (Table 3). Statically, the recovered isolates of *Salmonella* showed a significant difference in antibiotics resistance (F=13.283, P=0.0001). Phenotypically, 50% of recovered isolates showed XDR (extensively drug resistance) to eight classes. Multiple antibiotic resistance index (MARI) was very high, indicating high contamination.

4. Prevalence of virulence and antimicrobial resistance genes of *Salmonella* Typhimurium

Concerning *pef*A, *stn* and *hil*A as virulence genes, *stn* and *hil*A were detected in all tested *Salmonella* Typhimurium by 100% (2/2) while *pef*A was not detected (0/2). Furthermore, all the following antimicrobial resistance genes were detected by 100 % as bla_{TEM}, bla_{CTX-M}, bla_{SHV}, bla_{CMY}, *aad*A1, *tet*A, and *sul*1. While bla_{DHA} was not detected but *qnr*A was detected by 50%.

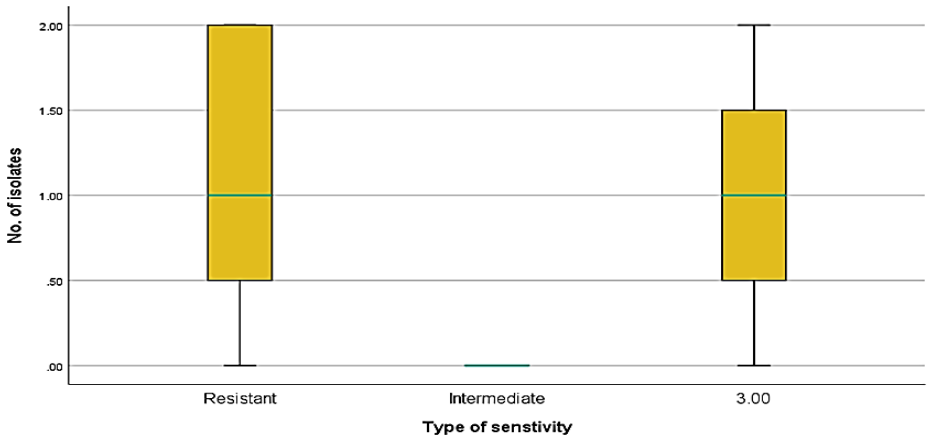

Figure (1). Box plot shows the pattern of sensitivity of *Salmone* Sensitive

Table 3. *The frequency of the phenotypic multidrug-resistance and the antibiotic-resistance (AR) genes among the Salmonella isolates (n=2)*

No. of isolates	Phenotypic resistant	Type of resistant	Multiple antibiotic resistance (MAR)
1	ampicillin, cefuroxime, cefotaxime, ceftazidime, gentamycin, streptomycin, ciprofloxacin, levofloxacin nitrofurantoin, tetracycline, and trimethoprim/sulfamethoxazole	XDR	0.75
1	ampicillin, cefuroxime, gentamycin, trimethoprim-sulfamethoxazole, tetracycline, nitrofurantoin	MDR	0.50

4. Discussion

The current study found a lower prevalence of *Salmonella* (2.7%) higher prevalence was detected by previous studies reported by ***Moon (2011), Ruban et al. (2010)*** *and* ***Abo hashem et al. (2022)*** who detected *Salmonella* in chicken meat by 38.33%, 31.99% and 8.3%, respectively. According to ***Fallah et al. (2013),*** a higher prevalence of *Salmonella* was found (44 %). While, lower prevalence rate of 0.94% was detected by ***Shekhar et al. (2013).*** *Salmonella* prevalence rates may range between studies due to a variety of factors, including geographic and seasonal variation, differences in sampling processes and sample sizes, and sanitary conditions during meat production and processing. According to the most recent data, *Salmonella* Typhimurium was found in 2.7% of samples. *Salmonella* Typhimurium at chicken meat can provide a health risk, especially if the meat is undercooked or contaminated with other meals.

Tetracycline, ampicillin, streptomycin, trimethoprim/sulfamethoxazole, and cefuroxime were all found to be resistant to *Salmonella* in this study; these results were consistent with ***Abo hashem et al. (2022),*** who found that *Salmonella* was resistant to amoxicillin and penicillin, ampicillin, gentamicin, and ciprofloxacin as well as streptomycin and trimethoprim. According to ***Hussain et, al. (2020),*** *Salmonella* isolates were also shown to be extremely resistant to oxytetracycline, ampicillin, amoxicillin, tetracycline, neomycin, and ciprofloxacin. Antimicrobial resistance genes bla_{TEM}, $bla_{\text{CTX-M}}$, and bla_{SHV} were found in 100% of *Salmonella* Typhimurium samples, which is consistent with the investigation findings by ***Hussain et al. (2020).*** According to the existing data, XRD (extensively drug resistant) was found in 50% of the

recovered isolates. The emergence and severity of XRD phenomena are both alarming and troubling. *hil*A gene were detected in all isolates which agreed with ***(Nayak et al., 2004; Dong et al., 2014).*** *hil*A gene, play a vital role in *Salmonella* virulence due to it encoded an OmpR/ToxR transcriptional regulator to activate the expression of invasion genes ***(Cardona-Castro et al., 2002).*** *stn* gene was prevalent among the isolated *Salmonella* by PCR, similar results have been reported by ***(Murugkar et al. 2003),*** who found that the chromosomally encoded virulent *stn* gene was widely distributed in all serovars.

In conclusion, in veterinary medicine, the overuse of antibiotics is a prime factor in the emergence of multidrug-resistant microorganisms. Antimicrobial agents should not be utilised as a substitute for proper hygiene standards, and alternative management strategies should be considered wherever possible. The use of antimicrobial agents by veterinarians should also be in accordance with local sanitary regulations.

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مدى انتشار ومدى ضراوة السالمونيلا تيفيموريوم ومدى مقاومتها للمضادات الحيوية فى لحوم الدجاج التى تباع بالتجزئة

ريهام الطرابيلى، محمد بلبل ، محمود عزت و مروة أبو هاشم

قسم البكتريولوجيا والمناعه والفطريات كلية الطب البيطرى– جامعة قناة السويس

الملخص العربي

تعتبر بكتريا السالمونيلا تيفيموريوم من اهم واكثر البكتريا المعزولة من الأمراض التي تنقلها الأغذية بشكل أساسي. تهدف الدراسه الى تحديد الانتشار العام للسالمونيلا في عينات لحوم الدجاج النيئة التى تباع بالتجزئة والتصنيف السيرولوجى لهذه العزلات واستخدام اختبار الحساسية لتحديد مدى مقاومه السالمونيلا تيفيموريوم للمضادات الحيوية المختلفة كما تهدف الدراسه الى تحديد بعض جينات الضراوة وجينات المقاومة للمضادات الحيوية المختلفة باستخدام تفاعل عديد البلمرة المتسلسل. تم تجميع عدد 75 عينه من لحوم الدجاج النيئة وباستخدام الاوساط البكتريولوجيه المختلفه تم زرع العينات واختبار المستعمرات المحتمل كونها سالمونيلا بالاختبارات البيوكيميكال المختلفه الخاصه بالسالمونيلا. حيث تم عزل السالمونيلا بنسبه 2.7% (75/2). تم تصنيف العزلتان سيرولوجيا على انهم سالمونيلا تيفيموريوم. باستخدام اختبار الحساسية للمضادات الحيوية المختلفه كانت العزلات مقاومة للتتراسيكلين (التتراسيكلين) والبنسلين (الأمبيسلين) والأمينوجليكوزيد (الجنتاميسين والستربتومايسين) والسلفوناميد (تريميثوبريم / سلفاميثوكسازول) والسيفالوسبورينات (سيفوروكسيم). فيما يتعلق بوجود جينات الضراوة فى العزلات اظهرت النتائج وجود بنسب 100%. *hil*A , *stn*
اما جينات المقاومه للمضادات الحيوية فقد تم تحديد تواجدها فى العزلات بنسبة 100% وذلك لكل من هذه الجينات. bla_{TEM}, bla_{CTX-M} and bla_{SHV}, bla_{CMY}, *aad*A1, *tet*A, and *sul*1
في الختام ، لحوم الدجاج النيئة ملوثة بشدة بالسالمونيلا التيفيموريوم. علاوة على ذلك ، كانت هناك أيضًا عوامل ضراوة مهمة في العزلات ، مما أدى إلى توسيع نطاق مخاوف الصحة العامة بسبب احتمالية التلوث المتبادل مع الأطعمة الأخرى وأكل الدواجن غير المطبوخة جيدًا