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Non-Typhoidal *Salmonella* in Chicken and Human: Prevalence, Serotyping, Antibiogram, Virulence, Antimicrobial Resistance Genes and *stn* Gene Sequencing

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Abstract | Non-typhoidal *Salmonella* is a zoonotic bacterium having a worldwide risk to public health and chicken industry. Primary aim of study is to assess *Salmonella* prevalence in chicken and human then serotyping, antibiogram, virulence and antibiotic resistance genes identification by PCR and sequence analysis. In total, 300 samples were gathered (100 chicken liver, 150 chicken feces, 50 human stool). Bacteriological examination, serotyping, antibiogram, virulence (*inv*A, *stn*) and antibiotic resistance genes (*erm*B, *aad*B) PCR screening for detection of virulence and antimicrobial resistance of strains and *stn* gene sequencing in *S.* Typhimurium of poultry (STP), *S.* Enteritidis of poultry (SEP) and *S.* Typhimurium of human (STH) were accomplished. *Salmonella* prevalence was 4% for both chicken (12/300) and human samples (2/50). Chicken isolates serotyped as *S.* Typhimurium (50%) and *S.* Enteritidis (50%). Human isolates typed as *S.* Typhimurium (100%, 2/2). All serovars were resistant to clindamycin and erythromycin (100%). Multidrug-resistance (MDR) was detected in SEP, STP, STH by 100%, 80% and 50%, respectively. *inv*A, *stn* genes were detected by 100% in all serovars. *erm*B was detected in SEP, STH and STP by 100%, 100% and 80% respectively. *aad*B gene was detected in SEP, STP and STH by 100%, 80% and 50%, respectively. *stn* sequencing revealed similarity between *S.* Typhimurium of human and *S.* Typhimurium and S. Enteritidis of chicken origin was 98.4% and 99.3%, respectively. It could be concluded that *S.* Typhimurium, *S.* Enteritidis of chicken and *S.* Typhimurium of human were MDR and share high *stn* gene similarity suggesting a public health alarm.

Keywords: *Salmonella*, Chicken, Serotyping, Antibiogram, virulence, Antibiotic resistance genes, Sequencing

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INTRODUCTION

Within the family Enterobacteriaceae, *Salmonella* is a prominent genus. Its species are motile, aerobic, facultative anaerobes, non-spore-forming, and gram-negative (Mahamedin *et al.*, 2024). An agglutination test using polyvalent antisera for somatic O antigens can be used to determine the serogroup of *Salmonella*. Afterwards, specific serovar can be identified by slide agglutination tests with monovalent antisera to certain O antigen and tube agglutination tests with antisera to flagella H antigens (Gast and Porter Jr, 2020).

Salmonellae are categorized into three primary classes according to the animals and humans they infect. The serotypes in the first group are unique to the human host; the serotypes in the second group are adapted to certain animal hosts; and the serotypes in the third group are not adapted and can cause diseases in in both a variety of animal species and humans (Ali and Sultana, 2015). The two main ways to contract nontyphoidal *Salmonella* are via eating contaminated food products sourced from animals and by coming into contact with animals. The most common human serovars of *Salmonella* are also found in poultry, which may indicate an epidemiologic link between chickens serving as a reservoir for people infections (Foley *et al*., 2011).

In Egypt, the prevalence of *Salmonella* spp. was 4.7% in chicken cloacal samples and 4.4% in human stool samples (Diab *et al*., 2019). The most frequently detected serotypes in chicken farms in Egypt were *Salmonella Enteritidis, S. Typhimurium, S. Shangani and S. Gueuletapee* (El-Sharkawy *et al*., 2017). Elkenany *et al*. (2019) detected the most common identified *Salmonella* serotype from chickens cloacal swabs in Egypt was *S. enteritidis* by 11.4% followed by *S. typhimurium* (8.6%). Salmonellosis is a costly disease in the chicken industry as it significantly impair performance, 24% broiler weight gain may dropping as well as 12% raising in food conversion ratio. Chicks are vulnerable to contracting *Salmonella* at an early age from a variety of sources. Over 200 *Salmonella* serovars were thought to be capable of colonizing a chicken's digestive system. *Salmonella* Typhimurium is an infectious serotype found in chickens and cause salmonellosis in humans. Antibiotics and immunizations are examples of preventive strategies used to combat this infection (Aljumaah *et al*., 2020).

Typhimurium is one of the most prevalent serotypes of *Salmonella enterica*, which still a significant worldwide contributor to foodborne illnesses in people. It is often derived from cattle, poultry, pigs, or their products (Sun *et al*., 2020). *S. typhimurium* (17.85%), *S. enteritidis* (14.28%) were the most prevalent serotypes detected in human stool in Egypt (Youssef *et al*., 2021) while *S. enteritidis* (39.4%) and *S. typhimurium* (24.1%), were the most prevalent serotypes detected in human stool in Ghana (Andoh *et al*., 2017).

Three mechanisms account for *Salmonella enterica* infections' pathogenicity: Colonization, intracellular survival, and host cell invasion. All these actions are regulated by the virulence genes present in *Salmonella* pathogenicity islands (SPI). The type III secretion system encoded in the SPI-1 is necessary for the most widespread invasion. Invasion protein A (*invA*) and other bacterial effector proteins are injected into cells of epithelium using this instrument, which resembles a needle. *inv*A is a virulence factor that has been studied in great details since infection to cross epithelial cells, it must first occur (Zermeño-Ruiz *et al*., 2022).

A combination of socioeconomic, environmental, and ecological factors, including the usage of antibiotics, have contributed to the the growing number of multidrug-resistant (MDR) bacteria, are the main causes of infectious disease development. Due to its high transmission capability, multidrug-resistant *Salmonella* (MDR) is becoming a greater threat and challenge. A few tactics that bacteria can employ to adapt to new surroundings and survive are virulence factors and antimicrobial resistance (AMR) (Sun *et al*., 2020). Two molecular methods are essential for characterizing pathogens: Sequencing and phylogenetic analysis (Azab *et al*., 2019).

The relentless usage of antibiotics may trigger the development of bacterial resistance, which could endanger public health by dispersing resistance from farm animals to people worldwide (CSE, 2014). Hence, the objectives of the study was to reveal *Salmonella* prevalence in chicken and human, serotyping of isolates, the antibiogram profiles, virulence (*inv*A, and *stn*) and antibiotic resistance genes (*erm*B and *aad*B) detection and sequence analysis of *stn* gene of *S. typhimurium* of chicken and human and *S. enteritidis* from poultry.

MATERIALS AND METHODS

Sample collection

Two hundred and fifty chicken samples (100 from liver, 150 from feces) were collected from different poultry farms at Ismailia government, Egypt. Fifty stool samples were collected from human at different Ismailia laboratories. Samples size was selected based on the large number of poultry farms in Ismailia compared to the number of human analysis laboratories. All samples were collected aseptically into buffered peptone water (Oxoid, UK), quickly sent as possible to the microbiology lab for identification bacteriologically.

Salmonella isolation and identification

For *Salmonella* isolation according to ISO (2002), pre-enrichment was performed in Rapport–Vassiliadis broth (Oxoid, Hampshire, UK), incubated for 18-24 hours at 37°C then cultivation on two selective media: xylose lysine deoxycholate (XLD) agar (HiMedia) and Salmonella–Shigella (SS) agar (HiMedia) for 18-24 hours at 37°C. Based on colonial characteristics, Gram stain and biochemical reactions (methyl-red, catalase test, oxidase test, citrate-utilization, indole, Voges-Proskauer, H2S production and urease), the expected *Salmonella* colonies were identified in accordance with ISO (2002).

Salmonella serotyping

Using *Salmonella* antiserum (Denka Seiken Co., Japan), *Salmonella* isolates were serotyped based on flagellar (H) and somatic (O) antigens using the scheme of Kauffman-White (Kauffman, 1974).

Salmonella antimicrobial susceptibility

Ten antibacterial agents (Oxoid Hampshire, UK): ciprofloxacin, trimethoprim/ sulfamethoxazole, oxytetracyclines, amoxicillin, gentamycin, erythromycin, ofloxacin, clindamycin, amoxicillin-clavulanic acid, and chloramphenicol have been tested on the isolates using the disk diffusion approach as outlined by Finegold (1988), antibiograms were carried out. The CLSI (2018) was followed in the interpretation of the results.

Virulence and antimicrobial resistance genes molecular determination

stn (*Salmonella* enterotoxin) and *inv*A (invasion protein A) virulence genes and *aad*B (aminoglycoside resistance gene) and *erm*B (macrolide resistance gene) antimicrobial resistance genes were detected by PCR. Following the directions provided by the manufacturer, the QIAamp DNA Mini kit (Qiagen, GmbH, Germany/Catalogue No. 51304) was utilized for DNA extraction. Every reaction included both positive (positive strains supplied by animal health research institute, Egypt) and negative controls (reactions without a DNA template). *Salmonella* virulence and antibiotic resistance gene DNA amplification was carried out in accordance with the references listed in Table 1. Using agarose gel electrophoresis 1.5% (w/v) (Applichem GmbH, Darmstadt, Germany) at 100 V in 1X TAE buffer (0.04 M Tris, 0.0001 M EDTA, pH 8.0) for 45 minutes, amplified PCR products were screened. Visualization was performed using 15 µL ethidium bromide (Sigma Aldrich, USA) and receive photographs using a UV transilluminator.

Sequencing

The *stn* gene was sequenced for three *Salmonella* isolates (one isolate from human, two isolates from poultry). Sequencing of a purified PCR product was performed using a ready-made Bigdye Terminator V3.1 cycle sequencing kit (Perkin-Elmer/Applied Biosystems, Foster City, CA) with Cat. No. 4336817 in both directions forward and reverse using an Applied Biosystems 3130 automated DNA sequencer (ABI, 3130, USA).

The initial step in proving sequence identity to GenBank accessions was, according to Altschul *et al.* (1990), a BLAST analysis (basic local alignment search tool). A comparative sequences analysis was conducted via the Clustal W multiple sequence alignment program, version 12.1 of MegAlign module of Lasergene DNA star software pairwise (Madison, Wisconsin, USA) which was designed by Thompson *et al.* (1994) and Phylogenetic analysis were executed through maximum likelihood, neighbour joining and maximum parsimony in MEGA6 (Tamura *et al.*, 2013).

Statistical analysis

All the data analysis were done by SPSS version 26.

RESULTS AND DISCUSSION

Phenotypic characteristics of *Salmonella* recovered from chicken and human

Salmonella showed colorless colonies with a black center on SS media, while exhibited red colonies with a black center on XLD media. *Salmonella* microscopically were identifiable as medium-sized, motile, non-sporulating, Gram-negative rods. Biochemical assays revealed positive results for citrate utilization, methyl-red, catalase and H2S production for all *Salmonella* isolates while indole, Voges-Proskauer, oxidase and urease were negative.

Salmonella prevalence in the examined samples of chicken and human

Ten *Salmonella* isolates were isolated from 250 bacteriologically investigated poultry samples with a prevalence of 4%. Five isolates from feces (3.3%) and five isolates from liver (5%). Moreover, two *Salmonella* isolates were isolated from 50 examined human stool samples with a prevalence of 4%. Table 2, Figure 1 show that there is

Table 1: Target genes, primer sequences, specific amplicon size of *Salmonella* virulence and antimicrobial resistance genes.

Gene type	Primer	Sequence	Amplified product	References
Virulence genes	*stn*	TTG TGT CGC TAT CAC TGG CAA CC	617 bp	Murugkar *et al.* (2003)
		ATT CGT AAC CCG CTC TCG TCC		
	*inv*A	GTGAAATTATCGCCACGTTCGGGCAA	284 bp	Oliveira *et al.* (2003)
		TCATCGCACCGTCAAAGGAACC		
Antimicrobial resistance genes	*aad*B	GAGCGAAATCTGCCGCTCTGG	319 bp	Frana *et al.* (2001)
		CTGTTACAACGGACTGGCCGC		
	*erm*B	GAAAAAGTACTCAACCAAATA	639 bp	Nguyen *et al.* (2009)
		AATTTAAGTACCGTTACT		

Table 2: The prevalence of *Salmonella* isolates in poultry and human samples.

Source of samples	Type of samples	No. of examined samples	No. of positive samples	Percentage of positive samples
Poultry	Feces	150	5	3.3%
	Liver	100	5	5%
Human	Stool	50	2	4%
p value			0.8	

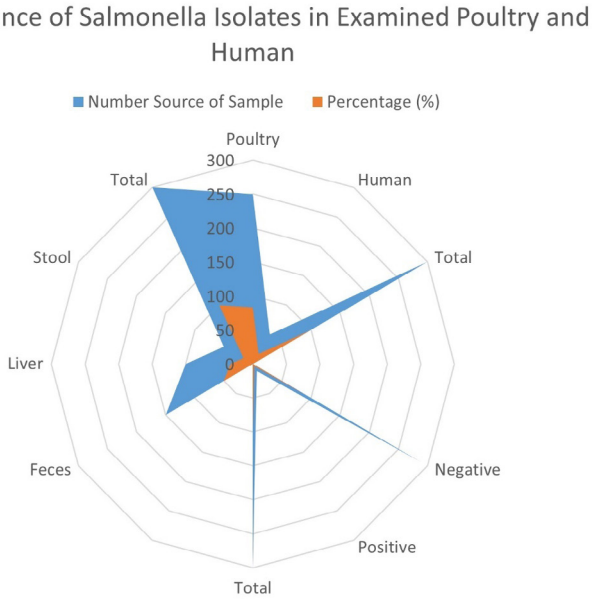

Figure 1: *Salmonella* prevalence in poultry and human samples

no statistically significant difference in the distribution of *Salmonella* isolates between the studied human and poultry samples (p > 0.05%).

The current study, detected 4% (10/250) total *Salmonella* prevalence in poultry and this was nearly just like Da Cunha-Neto *et al.* (2018) who reported *Salmonella* prevalence by 3.7% from chicken samples at Mato Grosso, Brazil. Moreover, lower prevalence was found by Rahmani *et al.* (2011) by 2.8% in Tehran, Iran and Dagnew *et al.* (2020) who found 2.9% *Salmonella* prevalence in poultry feces in Adama and Modjo towns, Ethiopia but Kayode *et al.* (2010), Mohammed and Dubie (2022) detected higher prevalence from chicken fecal samples in Ibadan, Nigeria and Addis Ababa, Ethiopia by 11%, 11.50%, respectively.

Concerning *Salmonella* prevalence in human stool, the following data detected it by 4% and it was the same recorded by Gharieb *et al.* (2015) and nearly just like Abate and Assefa (2021) who detected *Salmonella* prevalence by 4.8% in human stools in Ethiopia. Lower prevalence (2.8%) was detected by Dagnew *et al.* (2020) in Adama and Modjo towns, Ethiopia. Meanwhile, Tadesse (2014) proved higher prevalence of *Salmonella* recovered from human stool in Ethiopia (5.68%).

Serotyping of *Salmonella* isolates obtained from chicken and human samples

Ten *Salmonella* isolates isolated from poultry were serotyped as *S. typhimurium* (50%) and *S.* Enteritidis (50%). Concerning the two *Salmonella* isolates isolated from human were serotyped as *S.* Typhimurium (100%) as shown in Table 3. The following data proved that *S. typhimurium* and *S. enteritidis* were the serotypes recovered from poultry samples, the same results were reported by El-Kenany *et al.* (2019) who stated that *S. enteritidis* and *S. typhimurium* were the most commonly isolated serovars in poultry. *S. typhimurium* and *S. enteritidis* ability to infiltrate non-phagocytic cells and survive and multiply within macrophages and dendritic cells is what provides it its pathogenicity (Gal-Mor *et al.*, 2014). While Esaki *et al.* (2004) isolated only *S. enteritidis* from poultry. Certain strains of *Salmonella*, including S. enteritidis and *S. typhimurium*, pose a potential threat to public health as they are the primary sources of foodborne infections contracted from contaminated eggs and poultry products (Chlebicz and Slizewska, 2018).

Table 3: Number and percentage of different *Salmonella* serotypes (*n*=12; 10 from poultry, 2 from human).

Source of isolates	No. of tested isolates	Serotype	No. of *Salmonella* serotypes	Percentage of *Salmonella* serotypes
Poultry	10	*S. typhimurium*	5	50%
		S. enteritidis	5	50%
Human	2	*S. typhimurium*	2	100%

The current data detected *S. typhimurium* in human, this was confirmed by Andoh *et al.* (2017) who proved that the dominant serovars among hospital *Salmonella* isolates were *S. enteritidis* and *S. typhimurium* as. In most parts of the world, surveys have reported *S. enteritidis* and *S. typhimurium* as the major serovars found in humans (Saba *et al.*, 2013; Stevens *et al.*, 2008). *S. enteritidis* and *S. typhimurium* were the most commonly isolated from diarrheal illnesses in Ghana and the majority of other African nations (Bonkoungou *et al.*, 2013).

Antimicrobial sensitivity testing of *S. typhimurium*, *S. enteritidis* in chicken and *S. typhimurium* in human

In the following data, all *Salmonella* serovars from poultry and human were resistant to clindamycin and erythromycin by 100%. Ezzat *et al.* (2014) found that *Salmonella* serovars recovered from Dakahlia displayed excessive resistance to erythromycin. Additionally, Fagbamila *et al.* (2017) said that, all serotypes of *Salmonella* confirmed high resistance to erythromycin, (100%). When treating poultry infections,

Table 4: Antimicrobial sensitivity testing of *S. typhimurium* (n=5), *S. enteritidis* (n=5) in poultry and *S. typhimurium* (n=2) in human.

Antimicrobial class	Antibiotics	*S. typhimurium* (poultry)			*S. enteritidis* (poultry)			*S. typhimurium* (human)		
		Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Lincosamide	Clindamycin (DA)	0	0	5(100%)	0	0	5(100%)	0	0	2(100%)
Sulfonamide	Trimethoprim-sulfamethoxazole (STX)	4(80%)	1 (20%)	0	5(100%)	0	0	1(50%)	1(50%)	0
Tetracycline	Oxytetracycline (OTC)	3(60%)	0	2(40%)	2(40%)	2(40%)	1(20%)	1(50%)	0	1(50%)
Aminoglycoside	Gentamycin (GEN)	1 (20%)	0	4(80%)	0	0	5(100%)	1(50%)	0	1(50%)
Macrolide	Erythromycin (ERY)	0	0	5(100%)	0	0	5(100%)	0	0	2(100%)
Fluoroquinolone	Ofloxacin (OFL)	4(80%)	1 (20%)	0	5(100%)	0	0	1(50%)	1(50%)	0
	Ciprofloxacin (CIP)	5(100%)	0	0	5(100%)	0	0	2(100%)	0	0
Pencillin	Amoxicillin (AMX)	2 (40%)	2 (40%)	1(20%)	1(20%)	2(40%)	2(40%)	1(50%)	0	1(50%)
β-lactam-β Lactamase inhibitor combination	Amoxicillin/clavulanic acid (AMC)	5(100%)	0	0	4(80%)	1(20%)	0	2(100%)	0	0
Chloramphenicol	Chloramphenicol (CHL)	2 (40%)	2 (40%)	1(20%)	1(20%)	2(40%)	2(40%)	1(50%)	0	1(50%)
P Value		0.000			0.000			0.39		

erythromycin is the first drug that is advised to be administered. Subsequently, it is not taken for the appropriate length of time or at the suitable dose, the risk of acquiring antimicrobial resistance strains of bacteria against it is high (Shalaby *et al*., 2022).

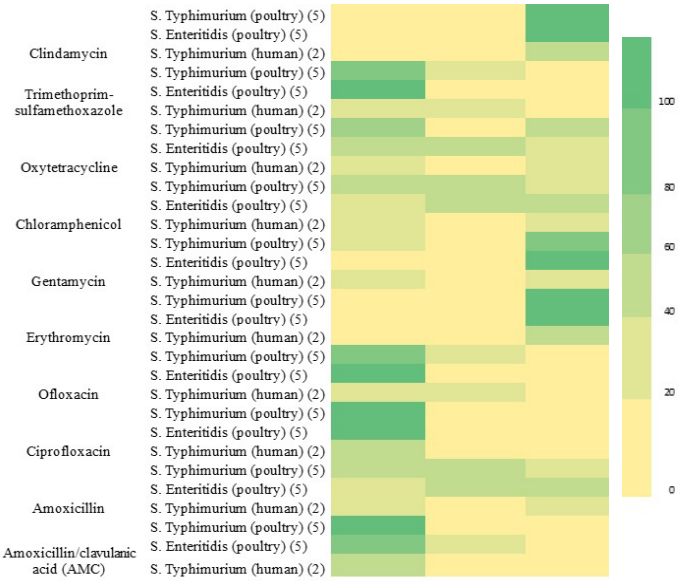

Figure 2: Heat map showed antimicrobial susceptibility of *S. typhimurium*, *S. enteritidis* in poultry and *S. typhimurium* in human.

All *Salmonella* serovars from poultry and human were sensitive to amoxicillin -clavulanic acid and ciprofloxacin by 100% as presented in Table 4 and Figure 2. Talukder *et al*. (2023) detected sensitivity of *Salmonella* to ciprofloxacin. Ciprofloxacin usage is regarded as the first medication line of preference (Gokul *et al*., 2010). *S. typhimurium* serovar isolated from poultry was resistant to gentamycin by 80% but was sensitive by 80% to sulfonamide and ofloxacin, 60% to oxytetracycline and 40% to chloramphenicol. Thung *et al*. (2016) who recorded penicillin and erythromycin resistance to while gentamicin, amoxicillin/ clavulanic acid, trimethoprim and tetracycline sensitivity. Meanwhile, Fagbamila *et al*. (2017) said that, all serotypes of *Salmonella* confirmed low resistance to gentamicin (5.9%).

S. enteritidis serovar isolated from poultry was resistant to gentamycin by 100% and was resistant to chloramphenicol and amoxicillin by 40% but was sensitive by 100% to trimethoprim-sulfamethoxazole and ofloxacin, 40% to oxytetracycline. These results were disagreed with El-Kenany *et al*. (2019) detected resistance of *S. enteritidis* by 100% against trimethoprim-sulfamethoxazole additionally, Mohammed and Dubie (2022) confirmed sensitivity to gentamycin (the highest level of antibiotic efficacy), ciprofloxacin and ampicillin by 95.7%, 78.3% and 69.6%. Nevertheless, tetracycline resistance was high (65.2%).

S. typhimurium serovar isolated from human was resistant to oxytetracycline, chloramphenicol, gentamycin and amoxicillin by 50% meanwhile, was sensitive to trimethoprim-sulfamethoxazole and ofloxacin by 50%. These findings nearly agreed with Andoh *et al*. (2017). Talukder *et al*. (2023) detected resistance against tetracycline (37.64%), trimethoprim/ sulfamethoxazole (32.92%), amoxicillin (32.18%). The expanding problem of antimicrobial resistance is caused by the misuse and/or

overuse of antibiotics throughout veterinary and human medicine (Gilchrist *et al*., 2007). It is commonly recognized that antimicrobial-resistant *Salmonella* in chicken and other food products will result in *Salmonella* resistance in people (Kagambèga *et al*., 2013). Statistically in this study, *S. typhimurium*, *S. enteritidis* of poultry showed a significant difference in their susceptibility patterns to the studied antimicrobial classes ($p < 0.05$) but *S. typhimurium* of human serovars exhibited no significant difference in their susceptibility patterns (p > 0.05%).

Virulence and antimicrobial resistance genes prevalence in *Salmonella typhimurium* and *Salmonella enteritidis*

In this data, *S. typhimurium* (2 strains from human, 5 strains from poultry) and *S. enteritidis* (5 strains from poultry) were screened for virulence (*inv*A, *stn*) and antimicrobial resistance genes (*aad*B, *erm*B) using PCR. Concerning *inv*A, *stn* virulence genes were identified by 100% overall the recovered strains of *S. typhimurium* and *S. enteritidis* as illustrated by Table 5. Shu *et al*. (2023) detected the same result of *inv*A, *stn* genes prevalence by 100% in all *Salmonella* serovars. *SPIs* genes enable nontyphoidal *Salmonella* to cause invasive illness, *inv*A is belong to SPI 1–5 in *Salmonella* (Kuang *et al*., 2015). Any strain of *Salmonella* can be identified in field samples by PCR using the distinct sequence found in the *stn* gene, which is present in all serotypes (Singh *et al*., 2017; Ammar *et al*., 2019). *inv*A contributes to cellular adhesion and invasion. *stn* plays a major role in the actual demonstration of pathogenic pathways (Murugkar *et al*., 2003).

Concerning, *erm*B antimicrobial resistance gene detection in the current data it was detected by 100% (5/5) of *S. enteritidis* of poultry origin, 80% (4/5) of *S. typhimurium* of poultry origin and 100% (2/2) of *S. typhimurium* of human origin. Shalaby *et al*. (2022) and Feng *et al*. (2024) were detected *erm*B gene in erythromycin resistant *Salmonella* isolates. The *erm*A, *erm*B, and *erm*C genes, control the emergence of erythromycin resistance (Gatermann *et al*., 2007).

Moreover, our results detected *aad*B antimicrobial resistance gene by 100% (5/5) in *S. enteritidis* of poultry origin, 80% (4/5) of *S. typhimurium* of poultry origin and 50% (1/2) of *S. typhimurium* of human origin as shown in Table 5 and Figure 3. Similarly, *aad*B resistance gene was detected by Doosti *et al*. (2016) and Ahmed *et al*. (2016). Gentamicin resistance phenotypically is caused by the *aad*B gene (Cameron *et al*., 1986). Statistically, the following study revealed no significant difference (p > 0.05%) in antibiotic resistance and virulence genes the distribution in poultry and human *Salmonella* serovars.

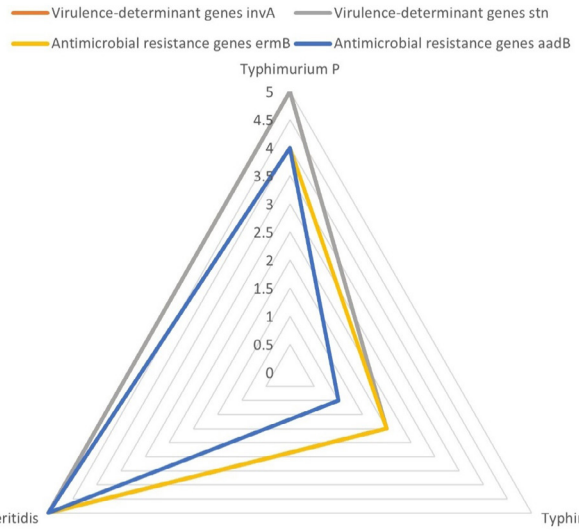

Figure 3: Prevalence of virulence and antimicrobial resistance genes in *S. typhimurium*, *S. enteritidis* of poultry and *S. typhimurium* of human.

Table 5: Virulence and resistance genes prevalence among *S. typhimurium* (*n*=5 from poultry, 2 from human) and *S. enteritidis* (*n*=5 from poultry).

p value	%	n	Bacterial serotype	Types of genes	
1.0	100	5	*S. typhimurium* of poultry origin	*inv*A	Virulence-determinant genes
	100	2	*S. typhimurium* of human origin		
	100	5	*S. enteritidis* of poultry origin		
	100	5	*S. typhimurium* of poultry origin	stn	
	100	2	*S. typhimurium* of human origin		
	100	5	*S. enteritidis* of poultry origin		
0.86	80	4	*S. typhimurium* of poultry origin	*erm*B	Antimicrobial resistance genes
	100	2	*S. typhimurium* of human origin		
	100	5	*S. enteritidis* of poultry origin		
	80	4	*S. typhimurium* of poultry origin	*aad*B	
	50	1	*S. typhimurium* of human origin		
	100	5	S. enteritidis of poultry origin		

Table 6: Prevalence of multidrug-resistance patterns and the resistance genes *Salmonella* serovars.

Serotype (source, No. of strains)	%	Type of resistance	Multidrug resistance profiles	Antimicrobial resistance genes	MAR Index
S. typhimurium (poultry, 4)	80	MDR	Three classes: Clindamycin, Gentamycin, Erythromycin	*erm*B, *aad*B	0.3
S. enteritidis (poultry, 5)	100	MDR	Three classes: Clindamycin, Gentamycin, Erythromycin	*erm*B, *aad*B	0.3
S. typhimurium (human, 1)	50	DR	Two classes: Clindamycin, Erythromycin	*erm*B	0.2
S. typhimurium (human, 1)	50	MDR	Four classes: Oxytetracycline, Chloramphenicol, Gentamycin, Amoxicillin	*erm*B, *aad*B	0.4

Salmonella serovars patterns of multidrug resistance

The results evidenced that 80% (4/5) of *S. typhimurium* of poultry and 100% (5/5) of *S. enteritidis* of poultry were MDR to three antimicrobial classes (Lincosamide: clindamycin, Aminoglycoside: Gentamycin, and Macrolide: erythromycin) and harbored *erm*B and *aad*B resistance genes. Moreover, 50% (1/2) of *S. typhimurium* of human were MDR to four antimicrobial classes (Tetracycline: oxytetracycline, Chloramphenicol: Chloramphenicol, Aminoglycoside: Gentamycin and Penicillin: Amoxicillin) and harbored *erm*B and *aad*B resistance genes. Nearly the same results were detected by Andoh *et al.* (2017) who revealed that 54.5% of *Salmonella* strains were multi-resistant. While, 50% (1/2) of *S. typhimurium* of human were drug resistant (DR) to two antimicrobial classes (Lincosamide: Clindamycin and Macrolide: Erythromycin) and harbored *erm*B resistance gene as shown in Table 6. The current investigation's MAR index values (0.2–0.4) revealed several patterns of resistance, suggesting that the *Salmonella* serovars originated from high-risk contamination.

Animal products containing antibiotic residues have the potential to cause antimicrobial drug resistance, as well as to enhance pathogenicity, virulence of the infection and lengthen and increase the cost of treatment. Humans are typically using more antibiotics every time, which raises the proportion of people who are resistant to antimicrobial medications. The capability to transfer resistance genes horizontally between *Salmonella* strains and other bacterial species exists. These other species may carry antibiotic resistance genes that aren't found in the *Salmonella* genetic pool (Hassan *et al.*, 2016).

Sequence analysis of *stn* virulence gene for *S. typhimurium* and *S. enteritidis* from poultry origin and *S. typhimurium* from human origin

One strain of *S. typhimurium* and one strain of *S. enteritidis* of poultry origin and one strain of *S. typhimurium* from human origin were sequenced for *stn* gene. Concerning, *S. typhimurium* and *S. enteritidis* of poultry origin the results revealed similarity (96.7-99.8%) of *stn* gene of the current *S. typhimurium* of poultry origin and other *Salmonella* serotypes of various origin. For example, the similarity was 97.6% - 99.8% for the following *Salmonella* serotypes: *S. typhimurium* SL1344 with Accession No. FQ312003, *S. typhimurium* ATCC 13311 with Accession No. CP009102, *S. typhimurium* SL1344RX with Accession No. CP011233, *S. typhimurium* SO_2 with Accession No. CP014356 and *S. typhimurium* LT2 (97.6 - 99.8%) with Accession No. AE006468. Also, the similarity was 98.6% - 98.8% for the following *Salmonella* serotypes: *S. enteritidis* ATCC BAA-708 with Accession No. CP025554, *S. enteritidis* EC20120916 with Accession No. CP007332, *S.* Enteritidis EC20110222 with Accession No. CP007323. Additionally, 97.6% - 99.5% similarity with *S. paratyphi* AATCC 9150 with Accession No. CP000026 and 97.7% - 99.3% similarity with *S.* Dublin RM099 with Accession No. CP117354.

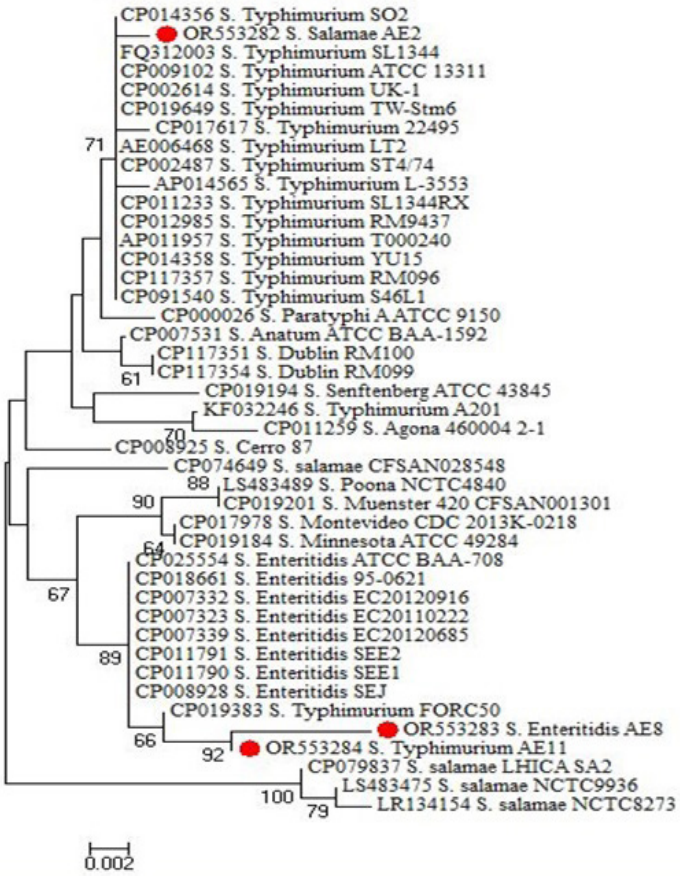

Figure 4: Phylogenetic tree illustrates the genetic relatedness between *stn* gene in *S. typhimurium* and *S. enteritidis* of the current data and *stn* gene of *Salmonella* serovars deposited in the GenBank. Red circles were used to highlight the current gene.

In case of *S. typhimurium* from human origin, the results revealed similarity (99.7%) of *stn* gene of the current *S. typhimurium* of human origin and other *Salmonella* serotypes of various origin. For example, the similarity was 99.7% for *S. typhimurium* FORC50 with Accession No. CP019383. Also, the similarity was 99.5% for the following *Salmonella* serotypes: *S. enteritidis* ATCC BAA-708 with Accession No. CP025554, *S. enteritidis* EC20120916 with Accession No. CP007332, *S. enteritidis* EC20110222 with Accession No. CP007323. The similarity was 98.3% for the following *Salmonella* serotypes: *S. typhimurium* SL1344 with Accession No. FQ312003, *S. typhimurium* ATCC 13311 with Accession No. CP009102, *S. typhimurium* SL1344RX with Accession No. CP011233. The similarity between the current *S. typhimurium* of human origin and the current *S. typhimurium* and *S. enteritidis* of poultry origin was 98.4% and 99.3%, respectively as shown in Figures 4 and 5.

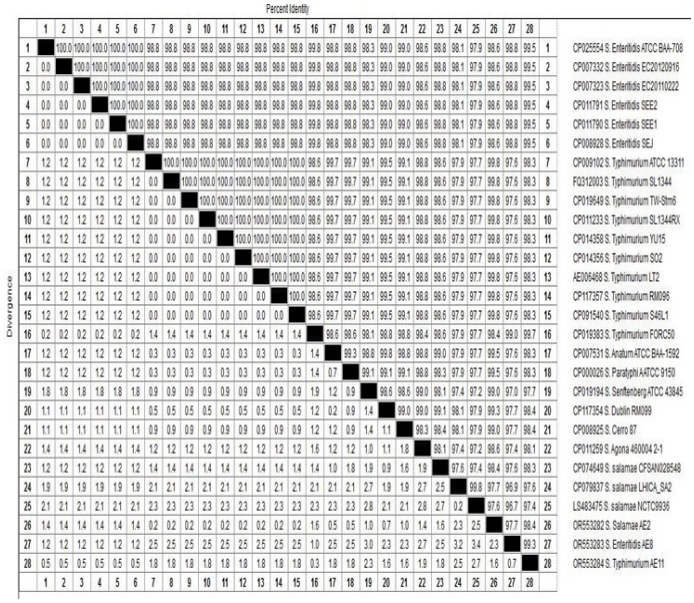

Figure 5: Reveals the percentage of the various *Salmonella* serovars' *stn* gene nucleotide sequence identities.

Similarity between the *stn* virulence gene of human *Salmonella* and the *stn* gene of poultry refers to the degree to which the genetic sequences and functions of the *stn* gene in both human and poultry *Salmonella* strains are alike. In both human and poultry *Salmonella*, this gene is involved in producing an enterotoxin that can disrupt the intestinal lining, leading to symptoms like diarrhea. Similarity between the genes suggests that *Salmonella* in both humans and poultry may share a common mechanism of causing illness, which can have implications for zoonotic transmission besides the development of strategies to maintain controlling *Salmonella* infections. High similarity would suggest that the pathogenic mechanisms used by *Salmonella* in poultry and humans are closely related, even if the strains infect different hosts.

CONCLUSIONS AND RECOMMENDATIONS

S. typhimurium and *S. enteritidis* were the serotypes detected in chicken samples while *S. typhimurium* was the *Salmonella* serovar found in human samples. All serovars were resistant to clindamycin and erythromycin. All serovars were harbored the virulence genes *inv*A and *stn*. *erm*B and *aad*B resistance genes were detected genotypically in serovars showed resistance against erythromycin and gentamycin phenotypically. *S. enteritidis*, S. typhimurium of chicken and S. typhimurium of human showed MDR against several antimicrobial classes (such as tetracycline, chloramphenicol, aminoglycoside and penicillin) suggesting a public health alarm. *stn* sequencing revealed similarity between *S. typhimurium* of human and *S. typhimurium* and *S.* Enteritidis of poultry origin that confirm presence of *stn* among many *Salmonella* serovars.

AUTHOR'S CONTRIBUTION

ME and MEA: The idea and study design. ME, FY, AE and MEA: Performed the experiments. AE and MEA: Drafted the manuscript. ME, FY, AE and MEA: Conducted the statistical analysis, data accuracy, and supervision. AE and MEA: Wrote and critically revised the manuscript. All authors have read and approved the final manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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