Research Article

Virulence and Antibiotic Resistance Profiles of *Salmonella* Isolated from Chicken Ready Meals and Humans in Egypt

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Abstract | Salmonellosis is a major public health concern with food economic losses worldwide. This study aimed to investigate the mutual role that may be played by chicken-ready meals and food handlers in the transmission of different *Salmonella* serotypes to hospitalized patients in Assiut Governorate, Egypt, as well as, to assess their pathogenic potential and antimicrobial resistance. Out of 150 chicken meals collected randomly from various restaurants and food shops including, shish-tawook, pane, and shawerma (50 for each), 10% were contaminated with *Salmonella* with the acquisition of shish-tawook (14%). On the other hand, 100 hand swabs that were assembled from food handlers working in the same places yielded 13 *Salmonella* isolates, at the time 4 isolates were only obtained from 50 hospitalized patients with diarrhea. From the public health point of view, *S. typhimurium, S. enteritidis,* and *S. kentucky* were serotyped from both food and human samples. Epidemiologically, insignificant sex risk factor was statistically found in this study although *Salmonella* was more common in males (14.67%) than in females (8%) among food handlers and the opposite among hospitalized patients (4.76% and 10.34% in males and females, respectively). *Salmonella* infection was dominant in 20 < 35 and 35 < 50 age groups among food handlers and patients, respectively. Complete resistance of the obtained isolates was showed to erythromycin, streptomycin, and nalidixic acid with the highest MAR index (0.640) appeared in clinical isolates from patients compared to food (0.517) and food handlers (0.471). All detected *Salmonella* serotypes harbored *inv*A gene through which a phylogenetic analysis was conducted for six isolates showing a high degree of similarity between them and those imported from Genbank. *hilA, spvC, stn*, and *qacED1* genes were detected in 75, 16.67, 66.67, and 50% of *Salmonella* serotypes, respectively. These findings signify the role played by chicken-ready meals, as well as their handling, in the high rate of multidrug-resistant *Salmonella* isolates and the risks it poses to public health.

Keywords | Chicken meals, *Salmonella*, Humans, Antibiotics, Virulence, Sequencing

Received | October 26, 2021; **Accepted** | November 28, 2021; **Published** | January 10, 2022
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Citation | Mubarak AG, Mustafa MM, Abdel-Azeem MW, Ali DN (2022). Virulence and antibiotic resistance profiles of *Salmonella* isolated from chicken-ready meals and humans in Egypt. Adv. Anim. Vet. Sci. 10(2): 377-388.
DOI | http://dx.doi.org/10.17582/journal.aavs/2022/10.2.377.388
ISSN (Online) | 2307-8316

INTRODUCTION

Salmonellosis remains a major public health issue worldwide with a huge global burden of morbidity and mortality especially in developing countries (Sodagari et al., 2020). It is estimated to cause 93.8 million human infections and 300,000 deaths annually (WHO, 2020) besides causing a major challenge in the global poultry industry. It is well known that human salmonellosis is associated with the consumption of different kinds of food, in particular poultry and poultry products (Favier et al., 2013). Other routes of infection between individuals are represented by the fecal-oral route and contact with infected pets through contamination of food and drink by the hands, thus disease outbreaks can occur (Munck et al., 2020).

Chicken meat and its products are characterized by deliciousness, nutritiousness, good flavor, and easily digested which make them very popular foods throughout the world, therefore poultry is a predominant source of foodborne illnesses (Chai et al., 2017). Over 2600 different *Salmonella* serotypes have been identified, 2000 of them can be found in chickens (Takaya et al., 2020). So, chickens have been implicated in most *Salmonella* outbreaks.

Salmonella recovered from chickens can be differentiated into three groups. The first group includes highly host-adapted and invasive serotypes such as *S. typhi* in humans, *S. gallinarum* and *S. pullorum* in poultry. The second is non-host adapted and invasive serotypes as *S. typhimurium*, *S. arizonae*, and *S. enteritidis*. The third group contains non-host adapted and non-invasive serotypes, which are mostly harmless to animals and humans (Andino and Hanning, 2015).

Salmonella pathogenicity has been related to many virulence genes existent in the chromosomal *Salmonella* pathogenicity islands (SPIs) (Nayak et al., 2004). The *inv*A gene is essential for epithelial cells invasion (El-Sharkawy et al., 2017) and has been established to be present in *Salmonella* species only, hence it is used in the genetic diagnosis of *Salmonella* species (Fekry et al., 2018). From medical and pharmaceutical points of view *invA* gene can help in developing specific medicines against *salmonella* (Almas et al., 2021). Whereas, an operon *spv* harbors five genes *spv*RABCD and is commonly associated with some serotypes initiating the systemic spread of the pathogen. The *spv*C is a virulence-related gene on the plasmid required for survival within the host cell (Card et al., 2016). *Hil*A gene is required to induce apoptosis of macrophages and invade epithelial cells (Borges et al., 2013). Besides, *Salmonella* enterotoxin (*stn*) gene which codes for enterotoxin production and is a causative agent of diarrhea (Xu et al., 2010).

Salmonellosis is often characterized clinically by nausea, vomiting, abdominal cramps, and diarrhea which is usually a self-limiting disease, but complications and deaths have been recorded especially in children, the elderly, and immunocompromised persons (Nayak et al., 2004). The increasing trend of *Salmonella* multi-drug resistance is mainly associated with the overuse of antibiotics in treating *Salmonella* infections and incorporation of growth promoters in animal feed (Ed-dra et al., 2017) causing a public health threat. In recent days, *Salmonella* strains showed increased resistance to several antibiotics comprising of β-lactams, cephalosporins, and non-β-lactam antimicrobials as tetracyclines, quinolones, sulfonamides, and polymyxins (das Neves et al., 2020; Rodrigues et al., 2020).

Meanwhile, resistance to the quaternary ammonium compounds (QACs) has been developed, which are cationic surface-active detergents, representing disinfectants of choice widely used in the poultry industry due to their low relative toxicity, good antibacterial properties, and non-corrosive to reduce or eliminate potentially pathogenic microbial loads (Haynes and Smith, 2003). The resistance to those disinfectants might be caused by intrinsic factors, with increased tolerance of the bacteria due to repeated exposure, or developed through genetic change. Likewise, there is evidence of the occurrence of cross-resistance and co-resistance between widely used disinfectants and antibiotics (Techaruvichit et al., 2016; Bakheet et al., 2017). The disinfectant resistance genes are commonly located in mobile genetic elements, four genes of QAC (*qac*E, *qacF*, *qacG* and *sugE* (*p*)) have been identified (Zou et al., 2014).

Therefore, this study aimed to assess the incidence, serotyping, virulence genes, and associations of *Salmonella* resistance recovered from chicken-ready meals, food handlers, and hospitalized patients in Assiut Governorate, Egypt.

MATERIALS AND METHODS

Study design and sampling

This study was conducted in Assiut Province during the period between 2018 and 2020 during which a total number of 150 chicken-ready meals were collected randomly from restaurants, food shops, and street vendors including shish-tawook, pane, and shawerma (50 for each). The samples were purchased in sterile and sealed plastic containers and transferred immediately to the laboratory for further processing. Twenty-five grams of each sample was added to 225mL of buffered peptone water (BPW) (Himedia, India) and mixed well by using a homogenizer, then incubated at 37 °C for 18–24 hours (Gracias and McKillip, 2004).

On the other hand, 150 human samples were collected, represented by 100 hand swabs which were collected from food handlers in the same restaurants and food shops, by dipping sterile cotton swabs into saline-containing sterile test tubes and then rubbing under fingernails, the palm of the hands, and between fingers. As well as 50 diarrhea samples from patients who suffered from gastrointestinal disturbances with diarrhea, who admitted to Abo-Noub Hospital, Assiut, Egypt were collected in clean cups. Then all samples were transferred to tubes contained BPW (Himedia, India) and incubated at 37 °C for 18–24 hours.

Isolation and identification of *Salmonella* (ISO, 2002)

Pre-inoculated BPW were transferred to 10 ml Rappaport-Vassiliadis Soya (RVS) broth (Himedia, India) as selective enrichment and incubated at 42˚C for 24 hours. Then a loopful was streaked on Xylose Lysine Deoxycholate (XLD) agar (Himedia, India) and incubated overnight at 37 ˚C. Typical colonies were picked and biochemically tested by standard devices as urease, sugar fermentation, methyl-red, Voges–Proskauer, indole, and citrate tests.

Serotyping of *Salmonella* isolates

Biochemically identified *Salmonella* isolates were serotyped to determine somatic (O) and flagellar (H) antigens using *Salmonella* antisera (Denka Seiken Co., Japan) according to Kauffman White scheme (Kauffman, 1974).

Phenotypic detection of antibiotic resistance

The antibiotic resistance test was performed using the disc diffusion method on Muller-Hinton according to the National Committee for Clinical Laboratory Standards (CLSI, 2017). The following antibiotics were assessed (µg/ml): Oxytetracycline (T, 30), Ciprofloxacin (CP, 5), Cephalothin (CN, 30), Neomycin (N, 30), Erythromycin (E, 15), Nalidixic acid (NA, 30), Ampicillin (AM, 10), Cephradine (CE,30), Doxycycline (DO,30), Kanamycin (K, 30), Streptomycin (S, 10), Cefotaxim (CF, 30), Gentamicin (G, 10), Amikacin (AK, 30), Sulphamethoxazol (SXT, 25), and Penicillin G (P, 10 IU).

Detection of some virulence genes

Salmonella serotypes obtained in this study were screened for the presence of some virulence and *qacED1* disinfectant genes using PCR. The primers used were presented in Table 1.

DNA extraction

Genomic DNA was extracted from *Salmonella* cultures using GeneJET Genomic DNA Purification Kit (Fermentas) following the manufacturer's instructions. The extracted DNA was stored at -20˚C till further use.

Amplification of virulence genes by Multiplex PCR

PCR amplification was performed using a thermal cycler (Master cycler, Eppendorf, Hamburg, Germany) following the manufacturer's instruction. The thermocycling conditions consisted of initial denaturation cycle at 94˚C for 2 min, 30 cycles of denaturation at 94˚C for 45 sec, annealing at 53˚C for 1 min, extension at 72˚C for 1 min and final extension at 72˚C for 7 min. Amplified DNA fragments were resolved by gel electrophoresis using 1.5 % (w/v) agarose stained with ethidium bromide solution (0.5µg/ml), visualized under an ultraviolet transilluminator and photographed.

Amplification of *qacED1* gene of *Salmonella*

The PCR cycling protocol was applied as following, An initial denaturation at 94˚C for 60 sec, followed by 35 cycles of denaturation at 94˚C for 60 sec, annealing at 64˚C for 30 sec and extension at 72˚C for 30 sec, followed by a final extension at 72˚C for 7 min. Finally, 5 µl of each amplicon was electrophoresed in 1% agarose gel stained with ethidium bromide, visualized and captured on UV transilluminator.

Sequencing *invA* gene

Purified PCR products using QIA quick extraction kit (Qiagen, Valencia, CA) of *invA* gene from six isolates; three of food origin (*S. kentucky*_CH1, *S. typhimurium*_CH2, *S. enteritidis*_CH3); two from hand swabs (*S. kentucky*_H1 and *S. typhimurium*_H2) and one from diarrhea (*S. enteritidis*_H3) were sequenced on an Applied Biosystems 3130 Sequencer (ABI, USA) using Bigdye Terminator V3.1 cycle sequencing kit (Perkin-Elmer). A BLAST® analysis (Basic Local Alignment Search Tool) (Altschul et al., 1990) was initially performed to establish sequence identity to GenBank. Purification of the sequence reaction occurred by using Centrisep (spin column), Cat. No. CS-901 of 100 reactions according to the manufacturer's instruction.

Phylogenetic analysis

The obtained sequences were subjected to BLAST similarity and phylogenetic analysis using the neighbor joining method on Mega 6 program (Tamura et al., 2013).

Statistical analysis

Data were statistically analyzed using a SPSS version 22, Pearson chi-square test, Fisher's exact test, and Monte Carlo test were used to predicate the association between variables followed by Contingency coefficient/ Phi correlation. Finally, Eta square (η2) was applied to measure the effect size of variance. To establish the risk factors, Mantel-Haenszel statistics were computed once for all variables and odd ratio between two dichotomous factor variables to measure the strength of the association.

RESULTS AND DISCUSSION

Incidence of *Salmonella* in chicken-ready meals

In this study, a series of devices were conducted for isolation, identification of *Salmonella* species and detection of their virulence and resistance. Out of the examined 150 chicken-ready meals, 15 (10%) were contaminated with *Salmonella* with the higher incidence in shish-tawook 14% (7/50)

followed by 10% (5/50) in panee, and the lowest incidence was detected in shawerma samples 6% (3/50) without significant difference (P=0.411), while a weak correlation between the chicken meals and *Salmonella* contamination (c=0.108) was found with small effect size (η2=0.012) as clarified in Table 2. Serotyping of the isolates revealed that *S. kentucky* and *S. typhimurium* were typed in the same percentage of 20 (3/15), also *S. enteritidis* and *S. molade* were equally serotyped as 13% (2/15), while *S. inganda, S. tamale, S. larochelle, S. tsevie*, and *S. wingrove* were typed as 7% (1/15) for each with statistically significant difference (^ =0.000) (Table 3).

Incidence of *Salmonella* in human samples

Data illustrated in Table 2 clarified that out of 100 and 50 examined hand swabs and diarrhea samples, 13 and 4 *Salmonella* isolates were obtained, respectively with insignificant association (P=0.362), and weak correlation (0.074^{P}). Seventeen human *Salmonella* isolates obtained in this study revealed six different serovars which significantly differ (P=0.000b) with *S. enteritidis* was the predominant 6 (35.3%), while *S. ttyphimurium* and *S. infantis* were the most frequent as 4 (24%) and 3 (18%), respectively. Whereas *S. kentucky, S. risen* and *S. heidelberg* represented as 1 (6%) for each, one untypable isolate was found (6%) from hand swabs as outlined in Table 4.

Table 1: Primer sequences of *Salmonella* genes.

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	Reference
invA	TATCGCCACGTTCGGCAA TCGCACCGTCAAAGGAACC	275	Nayak *et al.* (2004)
hilA	CGGAAGCTTATTTGCGCCATGCTGAGGTAG GCATGGATCCCCGCCGGCGAGATTGTG	854	Castro *et al.* (2002)
spvC	CGGAAATACCATCAAATA CCCAAACCCATACTTACTCTG	669	Swamy *et al.* (1996)
stn	TTGTGTCGCTATCACTGGCAACC ATTCGTAACCCGCTCTCGTCC	617	Murugkar *et al.* (2003)
qacED1	TAAGCCCTACACAAATTGGGAGATAT GCC TCC GCA GCG ACT TCCACG	62	Chuanchuen *et al.* (2007)

Table 2: Incidence of *Salmonella* in the examined samples.

Sources of samples	No. of examined samples	+ve *Salmonella* No. (%)	Pearson Chi-Square X^2 (P)	Contingency/Phi coefficient R (P)	Eta squared value (η2)	Odd ratio value
Chicken-ready meals						
Shish tawook	50	7 (14)	1.778 (0.411)	0.108^{C}(0.411)	0.012	0.392(0.095-1.613)
Panee	50	5 (10)				0.574(0.130-2.545)
Shawerma	50	3 (6)				Reference
Total	150	15 (10)				
Human samples						
Hand swab	100	13 (13)	0.829 (0.362)	0.074^{P} (0.362)	0.006	1.718 (0.530-5.571)
Diarrheal swab	50	4 (8)				
Total	150	17 (11.33)				
Over all total	300	32 (10.67)				

[C]: contingency coefficient; [P]: Phi coefficient.

Table 3: Serotyping of *Salmonella* isolates from chicken-ready meals.

Sources of samples	No. of isolates	*S. kentucky*	*S. typhimurium*	*S. enteritidis*	*S. molade*	*S. inganda*	*S. tamale*	*S. larochelle*	*S. tsevie*	*S. wingrove*	Fishers exact test	Eta squared
		No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No.(%)	No. (%)		
Shish-tawook	7	1 (14)	2 (29)	1 (2)	1 (14)	1 (14)	1 (14)	0 (0)	0 (0)	0 (0)	0.000^	
Panee	5	1 (20)	1 (20)	0 (0)	1 (20)	0 (0)	0 (0)	1 (20)	1 (20)	0 (0)	0.000^	
Shawarma	3	1 (33)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (33)	0.000^	0.841
Total	15	3 (20)	3 (20)	2 (13)	2 (13)	1 (7)	1 (7)	1 (7)	1 (7)	1 (7)		

^: fisher's exact test.

Table 4: Serotyping of *Salmonella* isolates from human samples.

Sources of sample	No. of isolates	*S. enteritidis*	*S. typhimurium*	*S. infantis*	*S. kentucky*	*S. rissen*	*S. heidelberg*	Untypable	Monte carlo test	Eta squared
		No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	Value (*P*)	
Hand swabs	13	5 (39)	2 (15)	2 (15)	1 (8)	1 (8)	1 (8)	1 (8)	72.535 (0.000b)	0.885
Diarrheal swabs	4	1 (25)	2 (50)	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)	26.398 (0.000b)	
Total	17	6 (35.3)	4(24)	3 (18)	1 (6)	1 (6)	1 (6)	1 (6)		

b: Monte Carlo test.

Table 5: Incidence of *Salmonella* in humans stratified by sex and age.

Variable	Hand swabs (No.=100)		Diarrheal swabs (No.= 50)		Fishers exact test	Mantel-haenszel odds ratio	Eta squared	Odds ratio
	No. of examined samples	+ve *Salmonella* No. (%)	No. of examined samples	+ve *Salmonella* No. (%)	P value	Value	Value	Value
Patient sex								
Male	75	11 (14.67%)	21	1 (4.76)	0.454	1.674 (0.464-6.035)	0.002	1.4 (0.465-4.211)
Female	25	2 (8)	29	3 (10.34)	1.000			
Patient age (years)								
5 < 20	7	0 (0)	16	1 (6.25)	1.000	1.388 (0.399-4.832)	0.000	Reference
20 < 35	63	10 (15.87)	20	2 (10)	0.721			2.2 (0.0185-26.157)
35 < 50	15	1 (6.67)	7	1 (14.29)	1.000			0.592 (0.122-2.864)
50 < 60	15	2 (13.33)	7	0 (0)	1.000			1 (0.128-7.812)

Data in Table 5 elucidated that *Salmonella* incidence in hand swabs differed with 14.67% among males in contrast to 8% among females, contrarily to its incidence in diarrhea samples which were 4.76% and 10.34% in males and females, respectively without significant values. Insignificantly, hand swabs collected from the age group of 20<35 showed the highest incidence of *Salmonellosis* (15.87%) followed by ages of 50< 60 (13.33%), finally the age group of 35<50 (6.67), while couldn't be detected in 5<20 age group. Patients from whom the highest positive diarrheal samples were collected ranged from 35<50 years old (14.29%) followed by 20<35 (10%) then 5<20 (6.25%). None of the diarrhea samples collected from the oldest age (50< 60) showed *Salmonella* infection.

Antibiotic resistance profile of food isolates

The results of *Salmonella* isolates resistance to sixteen antibiotics were given in Table 6. *Salmonella* isolates originated from chicken meals were all resistant to erythromycin and streptomycin (100%) followed by cephradine (93.3%), low frequency of resistance was observed to ampicillin (6.7%) and doxycycline (13.3%). As can be seen in Table 7, a high MAR index (0.125-1) was observed in *Salmonella* isolated from ready meals with the highest index value of 1 was found in one *S. Kentucky* isolate. Most *Salmonella* isolates were multi-drug resistant (MDR) to at least three antibiotics (erythromycin, streptomycin, and cephradine) with one *S. tamale* isolate was resistant to erythromycin and streptomycin only.

Table 6: Frequency of antibiotic resistance of *Salmonella* isolates.

Antibiotics	Food strains (No.=15)	Hand swabs strains (No.=13)	Diarrheal strains (No.=4)
	No. (%)	No. (%)	No. (%)
Erythromycin (E)	15(100)	12 (92.3)	4 (100)
Streptomycin (S)	15 (100)	13 (100)	4 (100)
Cephradine (CE)	14 (93.3)	12 (92.3)	3 (75.0)
Sulphamethoxazol (SXT)	12 (80)	7 (53.8)	3 (75.0)
Cephalothin (CN)	11 (73.3)	6 (46.1)	3 (75.0)
Nalidixic acid (NA)	9 (60)	9 (69.2)	4 (100)
Cefotaxim (CF)	8 (53.3)	5 (38.5)	3 (75.0)
Penicillin G (P)	6 (40)	8 (61.5)	3 (75.0)
Neomycin (N)	6 (40)	4 (30.8)	3 (75.0)
Oxytetracycline (T)	6 (40)	6 (46.1)	2 (50.0)
Kanamycin (K)	6 (40)	4 (30.8)	2 (50.0)
Gentamicin (G)	5 (33.3)	1(7.7)	1 (25.0)
Amikacin (AK)	5 (33.3)	2 (15.4)	2 (50.0)
Ciprofloxacin (CP)	3 (20)	3 (23.1)	1 (25.0)
Doxycycline (DO)	2 (13.3)	4 (30.8)	2 (50.0)
Ampicillin (AM)	1 (6.7)	2 (15.4)	1 (25.0)

Antibiotic resistance profile of human isolates

Table 6 showed that all *Salmonella* isolates obtained from food handlers pronounced a complete resistance to streptomycin with higher resistance to erythromycin and cephradine (92.3%). Contrarily, gentamicin was the most effective against *Salmonella* followed by amikacin and ampicillin with resistance rates of 7.7, 15.4, and 15.4%, respectively. One *S. enteritidis* isolate exhibited MAR index of 1, all isolates were MDR to three antibiotics (erythromycin, streptomycin, and cephradine) or more as clarified in Table 8.

About diarrheal isolates, Table 6 displayed a complete resistance to erythromycin, streptomycin, and nalidixic acid followed by cephradine, sulphamethoxazol, cephalothin, cefotaxim, penicillin G, and neomycin with the same resistance rate of 75.0%, low level of resistance was detected to gentamicin, ciprofloxacin, and ampicillin (25.0%). Constantly, data recorded in Table 9 exposed that one *S. typhimurium* isolate showed MAR index of 1 with MDR in all isolates exhibiting resistance to three antibiotics (erythromycin, streptomycin, and nalidixic acid) or more.

Table 7: Antibiotic resistance profile of food *Salmonella* isolates.

No.	*Salmonella* serotype	Antimicrobial resistance profile	MAR index
1	*S. kentucky*	E, S, CE, SXT, CN, NA, CF, P, N, T, K, G, AK, CP, DO, AM	1
2	*S. kentucky*	E, S, CE, SXT, CN, NA, CF, P, N, T, K	0.688
3	*S. kentucky*	E, S, CE	0.188
4	*S. enteritidis*	E, S, CE, SXT, CN, NA, CF, P, N, T, K, G, AK, CP, DO	0.938
7	*S. enteritidis*	E, S, CE, SXT	0.250
8	*S. typhimurium*	E, S, CE, SXT, CN, NA, CF, P, N, T, K, G, AK, CP	0.875
9	*S. typhimurium*	E, S, CE, SXT, CN, NA, CF	0.438
9	*S. typhimurium*	E, S, CE, SXT, CN, NA, CF	0.438
10	*S. molade*	E, S, CE, SXT, CN, NA, CF, P, N, T, K, G, AK	0.813
11	*S. molade*	E, S, CE, SXT, CN	0.313
12	*S. wingrove*	E, S, CE, SXT, CN, NA, CF, P, N, T, K, G, AK	0.813
13	*S. larochelle*	E, S, CE, SXT, CN, NA	0.375
14	*S. tsevie*	E, S, CE, SXT, CN	0.313
15	*S. inganda*	E, S, CE	0.188
16	*S. tamale*	E, S	0.125
Average 0.517			

MAR index= No. of resistance / Total No. of tested antibiotics.

Table 8: Antibiotic resistance profile of hand swabs *Salmonella* isolates.

NO	*Salmonella* strains	Antimicrobial resistance profile	MAR index
1	*S. enteritidis*	S, CE, E, NA, P, SXT, T, CN, CF, K, N, DO, CP, AM, AK, G	1
2	*S. enteritidis*	S, CE, E, NA, P, SXT, T, CN, CF	0.563
3	*S. enteritidis*	S, CE, E, NA, P, SXT, T, CN	0.500
4	*S. enteritidis*	S, CE, E, NA, P	0.312
5	*S. enteritidis*	S, CE, E	0.187
6	*S. typhimurium*	S, CE, E, NA, P, SXT, T, CN, CF, K, N, DO, CP, AM, AK	0.938
7	*S. typhimurium*	S, CE, E, NA, P, SXT	0.375
8	*S. infantis*	S, CE, E, NA, P, SXT, T, CN, CF, K, N, DO, CP	0.812
9	*S. infantis*	S, CE, E, NA	0.250
10	*S. kentucky*	S, CE, E, NA, P, SXT, T, CN, CF, K, N, DO	0.750
11	*S. heidelberg*	S, CE, E	0.187
12	*S. rissen*	S, CE, E	0.187
13	Untypable	S	0.062
Average 0.471			

Table 9: Antibiotic resistance profile of diarrhea *Salmonella* isolates.

No.	*Salmonella* strains	Antimicrobial resistance profile	MAR index
1	*S. typhimurium*	S, E, NA, P, CE, CF, N, SXT, CN, K, T, AK, DO, AM, G, CP	1
2	*S. typhimurium*	S, E, NA	0.187
3	*S. enteritidis*	S, E, NA, P, CE, CF, N, SXT, CN, K, T, AK, DO	0.812
4	*S. infantis*	S, E, NA, P, CE, CF, N, SXT, CN	0.563
Average 0.640			

Distribution of some virulence and *qacED1* genes among *Salmonella* isolates

A representative detection of some *Salmonella* virulence and *qacED1* disinfectant genes were exposed in Table 10 revealing that all 12 *Salmonella* serotypes harbored *inv*A gene, while *hilA, spvC*, *stn*, and *qacED1* genes were detected in 75, 16.67, 66.67, and 50% of the serotypes, respectively with the acquisition of *S. typhimurium* which contained all examined genes (Figures 1, 2).

Table 10: Incidence of detected virulence genes from obtained *Salmonella* serotypes.

Virulence genes/ *Salmonella* sertypes	invA	hilA	spvC	Stn	qacED1
S. kentucky	+	+	-	+	-
S. typhimurium	+	+	+	+	+
S. enteritidis	+	+	-	+	+
S. molade	+	+	-	-	-
S. wingrove	+	-	-	+	-
S. larochelle	+	+	-	-	-
S. inganda	+	+	-	-	+
S. infantis	+	+	-	+	-
S. tsevie	+	+	-	+	+
S. tamale	+	-	-	+	-
S. heidelberg	+	+	-	+	+
S. rissen	+	-	+	-	+
Total isolates (No.=12)	100%	75%	16.67%	66.67%	50%

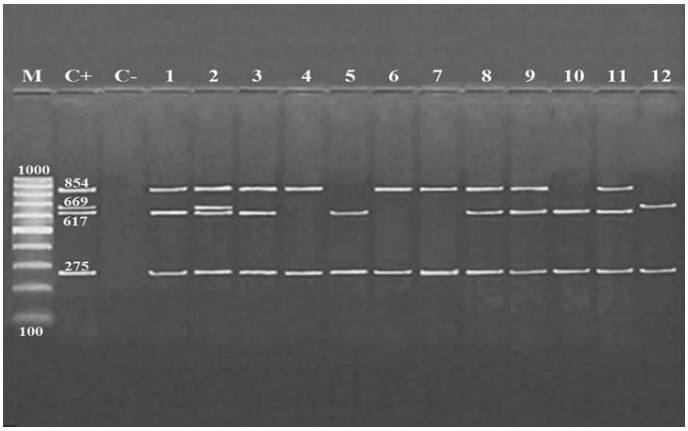

Figure 1: Agarose gel electrophoresis of multiplex PCR of *invA* (275 bp), *stn* (617 bp), *spvC* (669 bp) and *hilA* (854 bp) as virulence genes for characterization of *Salmonella* species.

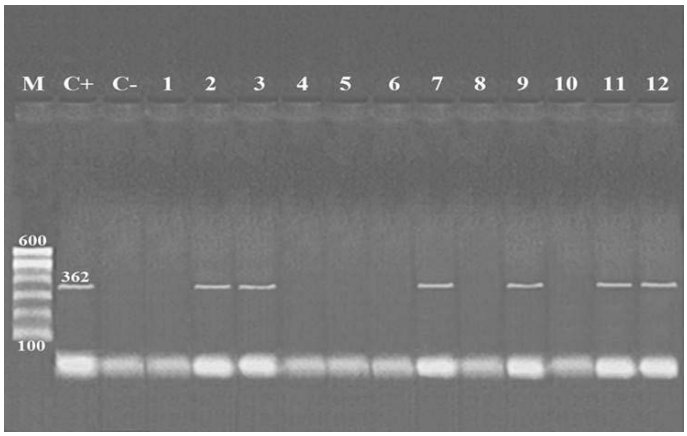

Figure 2: Agarose gel electrophoresis of PCR of *qac*ED1 (362 bp) gene for characterization of *Salmonella* species.

Phylogenetic analysis

Multiple sequence alignment and phylogenesis revealed a high degree of similarities between the local isolates (CH1, CH2, CH3, H1, H2, and H3) obtained from chicken-ready meals and human samples, and those retrieved from the GeneBank (Figure 3).

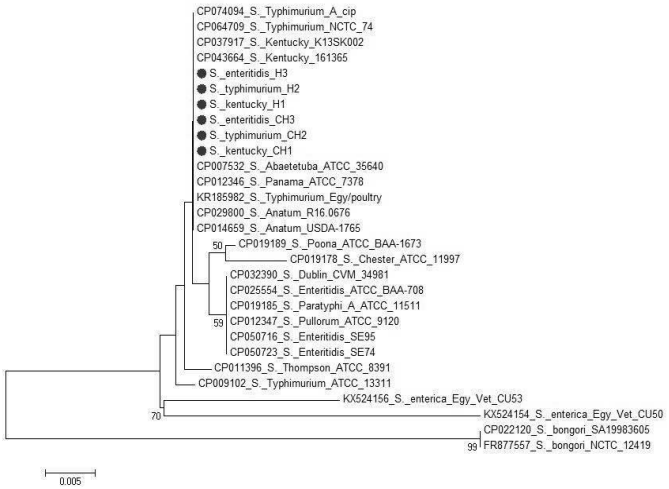

Figure 3: Phylogenetic tree of *Salmonella* isolated from chicken-ready meals and humans based on *invA* gene.

Poultry meat is one of the frequent vehicles of salmonellosis as a zoonotic infection especially ready to eat chicken meals which is in high demand as a result of their high biological value, reasonable price, and easy served. Through our study, *Salmonella* species were detected in 10% of the examined chicken meals with the acquisition of shish-tawook (14%)

which may be attributed to the fact that the temperature of grilling is not sufficient to kill micro-organisms, besides it receives more handling during preparation. This result was consistent with Abd El-Tawab et al. (2015) and Mustafa et al. (2021) who found that 10.9% and 10% of the examined chicken meat were positive for *Salmonella*, respectively. Other studies registered higher incidences of the organism as those conducted by Hassanin et al. (2014) (22.2%) and Saad et al. (2015) (15%). In contrarily, Medeiros et al. (2011); Akbar and Anal (2015) recorded lower incidences of 2.7% and 0.55%, respectively. Contamination of ready meals with *Salmonella* might be attributed to low slaughter hygiene and cross-contamination of the products at different stages of chicken dressing and preparation in the retail shops. The variation in *Salmonella* incidence may be due to the differences in manufacturing practices, handling from producers to consumers and the effectiveness of hygienic measures applied during production.

Nine *Salmonella* serovars were isolated in this study from chicken meals which are all pathogenic to humans. *S. typhimurium* and *S. kentucky* were the predominant serotypes (20%). In another study conducted by Elkenany et al. (2019), *S. enteritidis* was the most common identified serotype followed by *S. typhimurium*, and *S. kentucky*. Also, Siddique et al. (2021) discovered that *S. typhimurium* and *S. enteritidis* were the predominating types.

Controversially, there is a marked increase in antimicrobial resistance levels in developing countries as access to antimicrobials is easy and somewhat can be bought without prescription which leads to indiscriminate and widespread uses of antimicrobials both in the veterinary and public health practices (Henton et al., 2011; OIE, 2011). By evaluating antibiotic resistance in ready meals isolates, complete phenotypic resistance against erythromycin and streptomycin antibiotics with a higher resistance to cephradine (93.3%) and sulphamethoxazol (80%) were deemonsstrated which are commonly used in veterinary and human medicine, so considered alarming. Approximate susceptibility to ampicillin, doxycycline, and ciprofloxacin has been detected. As a result, more attention is needed towards foodborne pathogens control. In concordance with our results, Akbar and Anal (2015) found that all isolates from chicken sources were resistant to streptomycin. Siddique et al. (2021) recorded complete resistance to erythromycin and streptomycin. Contrarily, Moura et al. (2018); Perin et al. (2020) detected a high level of resistance to amoxicillin and ceftriaxone.

In the meantime, a high MAR index ranged from 0.125–1 has been observed in different food *Salmonella* serovars in our study, similarly, Siddique et al. (2021) detected a high index of 0.62–0.91.

Regarding human cases, unexpectedly, our results showed a higher percentage of the microbe in food handlers hand swabs (13%) than in patients diarrhea (8%). So, this highlights the role played by food handlers in the transmission of food-borne diseases that represent a global health burden and they must always be under oversight. Variable incidences in poultry shops workers and food handlers were reported by Salem et al. (2017) and Yesigat et al. (2020) as 20% and 2.5%, respectively. On the other hand, Rabie *et al*. 2012 recorded a closely related incidence of salmonellosis in patients diarrhea (10%), while higher incidences were declared by Salem et al. (2017) (13.79 %) and Ngogo et al. (2020) (16.5%).

Detection of *Salmonella* in food handlers is usually associated with untrimmed fingernails under which the bacteria locate, thus they might play a great role in the food-borne spread of salmonellosis, the result that confirmed with Phi coefficient statistic. Serotyping declared that numerous serovars could be recovered from the examined hand swabs including, *S. typhimurium, S. infantis, S. kentucky, S. rissen, S. heidelberg* with the acquisition of *S. enteritidis*. While one isolate couldn't be serotyped. From diarrheal samples, *S. Enteritidis, S. infantis* with the obsession of *S. typhimurium* have been recorded. Therefore, *S. enteritidis* was the dominant serotype (35%) in the overall human samples followed by *S. typhimurium* (24%) and *S.* Infantis (18%). Proportionate with our result, Qi et al. (2019) and Chirambo (2020) revealed that *S. enteritidis* and *S. typhimurium* are the most common serotypes causing gastroenteritis reflecting the importance of the results obtained in this study.

A total of 150 individuals participated in this study represented by 100 food handlers and 50 hospitalized patients, at ages ranging from 5-60 years old. The majority of the participants were between 20 and 35 years. Insignificant sex risk factor was found in the current study despite the difference in the infection rate between males and females as 14.67, 8 and 4.76 and 10.34% in food handlers and patients, respectively. Compatible results were achieved by Mengist et al. (2018) and Ngogo et al. (2020). This can be explained by the fact that the incidence of infection increases with increased contact with food either among food handlers or patients. Consistent with our findings, several studies have shown that the incidence of diarrheal illness, in general, is higher in women than men.

Concerning age, a high level of *Salmonella* infection was reported at ages ranging from 20 < 35 years among food handlers, which may be due to that this is the right age for working and so more contact with infection sources. Also, may be as a consequence of their substandard personal hygiene and lack of washing hands especially after using

the toilet. The same result was obtained by Mengist et al. (2018) with the highest infection level at ages of 21-30 years. Belonging hospitalized patients, the age group of 35<50 showed the highest salmonellosis level which may be attributed to the feeding of undercooked foods or food contaminated after cooking during preparation or serving. Similar data was recorded by Chirambo (2020). Contrarily, patients at ages of 11-20 years exhibited the highest degree of infection followed by 21–30 age group by Teshome et al. (2019).

In the current study, human *Salmonella* isolates showed complete or higher resistance to erythromycin, streptomycin, nalidixic acid, cephradine, cefotaxim, sulphamethoxazol, and penicillin G which may be affiliated to the unrestricted use of these antibiotics in the community. Our results were compatible with those obtained by Maripandi and Al-Salamah (2010) and Singh et al. (2012). While somewhat differ from results recorded by Mengist et al. (2018) and Yesigat et al. (2020) who detected complete resistance to ampicillin. The increased resistance pattern showed by *Salmonella* population remains a serious public health problem and could be responsible for treatment failures in some clinical cases.

Quinolones are broad-spectrum antibiotics used in the treatment of several infections including salmonellosis particularly in the elderly and immunosuppressed patients which represented in our study by complete resistance of the human isolates to nalidixic acid while were sensitive to ciprofloxacin. High resistance to cefotaxim, the drug of choice when quinolones are contraindicated (Egorova et al., 2008), also was reported by this study giving warning about the use of antibiotics.

Salmonella virulence is influenced by antimicrobial and disinfectant resistance, as well as the presence of virulence genes. So, 12 *Salmonella* serotypes were analyzed for the presence of four virulence genes; *inv*A, *hilA, spvC,* and *stn* in addition to *qacED1*. In all *Salmonella* isolates, *invA* gene was detected explaining their ability to invade and so causing gastroenteritis (Lan et al., 2018). Several studies reported the detection of this gene in all *Salmonella* species as their inner membranes contain protein coded for by *invA* (Amini et al., 2010; Ramatla et al., 2020).

Nine *Salmonella* isolates in the present study harbored *hilA* gene (75%) which activates the expression of *invA* gene, this result was in corroboration with Borges et al. (2013) who found that all *Salmonella* isolates were positive for *invA* and *hilA*. 16.67% of the obtained isolates contained *spvC* gene, contrasting results were obtained by Soto et al. (2006) who detected it in all *Salmonella* isolates unlike Chaudhary et al. (2015) who could not detect that gene. The chromosomally encoded virulent *stn* gene is widely distributed among *Salmonella* serovars, this data agrees with the current work since it could be detected in a percentage of 66 of the obtained isolates. Similar results were reported by Murugkar et al. (2003) and Ezzat et al. (2014).

The *qacED1* gene was detected by 50% of the isolates, Abd El-Tawab et al. (2016) distinguished *qacED1* gene in 57.14% of *Salmonella*. While Nabil and Yonis (2019); Iraqi et al. (2020) detected that gene in all *Salmonella* representative isolates and also found a significant association between the presence of *qacED1* and antimicrobial resistance.

A high homology between CH1, CH2, CH3, H1, H2, and H3 which were obtained from chicken meals, food handlers, and patients was accentuated based on *invA* gene sequencing, which is a very important tool for periodical evaluation of mutagenicity compared with the published sequences on GenBank. This explains the role that chicken-ready meals and food handlers play in transmitting salmonellosis to patients.

CONCLUSIONS AND RECOMMENDATIONS

This work presented a comprehensive study of *salmonella* presence in chicken ready meals as a major concern for human salmonellosis. The close evolutionary relationship between isolates in our study highlights the potential role of food handlers in transmitting different *Salmonella* serotypes to ready meals during preparation as well as to customers. Furthermore, resistance of most recovered *Salmonella* isolates to multiple antibiotics is of great priority. Such data impose screening of food handlers, training of hand hygiene practices, and regular monitoring of food handling practices to avoid diseases that can be acquired through improper food handling, like salmonellosis. In addition, bio-control measures must be applied to control *salmonella* infection within chicken farms and antibiotic resistance must be managed through enforcement of management strategies.

ACKNOWLEDGMENTS

We would like to thank the staff members of Abo-Noub Hospital for helping in the collection of patients samples as well as, workers and food handlers.

NOVELTY STATEMENT

This research sheds light on the role that food handlers play in the spread of multi-drug resistant Salmonella species through chicken-ready meals, as few studies have

addressed the issue from a zoonotic point of view.

AUTHOR'S CONTRIBUTION

All authors contributed equally and approved the final manuscript.

Ethical approval

This study was approved by the South Valley University ethical committee, Qena, Egypt (No. 21/15.9.2019). Also, Oral consent was obtained from each participant.

Conflict of interest

The authors have declared no conflict of interest.

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