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Characterization of *Salmonella* spp. Isolated From Poultry Giblets, Calves and Human Beings in Menoufiya Governorate

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ABSTRACT

Salmonellosis remains as an important public health problem and of economic importance worldwide. The aim of this study was to indicate the epidemiology of salmonellosis in both animal and human being in Menofiya Governorate. A total of 352 samples from poultry giblets (52 liver, 32 gizzard, 30 heart), frozen minced meat (58), calves faeces (50) and 130 stool samples were collected and examined bacteriologically, biochemically. Our results revealed that the prevalence of *Salmonella* spp. was 71.15% (37/52), 59.4% (19/32), 60% (18/30), 24.14% (14/58), 6% (3/50) and 42.3% (55/130) in liver, gizzard, heart, frozen minced meat, calves fecal matter and human stool, respectively. Moreover, 10 isolates of them had been serotyped and for further confirmation 33 isolates from these sources were examined using PCR to detect presence of *Salmonella* species *inv*A gene and virulence genes (*mgt*C and *hil*A). By serotyping *S.typhimurium* was detected in liver, heart, frozen minced meat and calves fecal matter, *S.enteritidis* was found in gizzard and human stool samples, while *S.kentucky* was only detected from human stool samples. Furthermore, PCR on 33 isolates detected (100%) for *inv*A, (84.85%) for *mgt*C and (90.9%) for *hil*A gene. In conclusion, *Salmonella* species has potential zoonotic spread and the presence of virulence genes in isolates from animals, products and in-contact human being, confirming the role of food from animal origin in transmission of salmonellosis.

Keywords: *Salmonella* spp., *XLD, Serotyping*, *inv*A, *hil*A, *mgt*C.

INTRODUCTION

Salmonellosis is a food-born infection of worldwide importance (Ricci, 2003) and it is a zoonotic bacterial disease of national and international health and economic importance , its distribution often matches the patterns of animal's trade products and food and the migration way of humans and animals (Gilbert *et al.*, 2010).

Salmonella species Infection has an estimation as 1.3 billion incidence of non-typhoidal salmonellosis worldwide each year (Coburn *et al.*, 2007) and is estimated to cause more than 1.2 million illnesses each year in the United States, with more than 23,000 hospitalizations and 450 deaths (CDC, 2011).

As it cause major public health and economic problems in both developed and developing

countries. *Salmonella* species are the second most reported bacteria causing food-borne disease in human, following *Campylobacter* species (EFSA, 2010). More than 2610 *Salmonella* serovars were recognized and almost all of them are able to cause illness in animals and human (Guibourdenche *et al.,* 2010).

S. Enteritidis and *S.* Typhimurium are the most frequently reported serotypes causing human salmonellosis in both the EU and the United States, while the incidence of *S.* Infantis is increasing. This confirms the need for improving the prevention and control of *Salmonella* species in food industry (Miya *et al.,* 2014 and Djordjvić *et al.,* 2018).

*S.*Kentucky currently is considered among top ten serovars causing gastroenteritis in humans (Bonalli *et al.,* 2012). This serovar was reported from poultry sources (Boyle *et al.,* 2010).

Salmonella infection can be detected through identifying the presence of the bacteria or identification and quantification of antibodies to *Salmonella* in the animal. The gold standard for *Salmonella* detection is bacteriological culture. Some common methods used in addition to culture are serotyping (Hendriksen, 2003).

PCR has been developed to detect specific genes important in the virulence of micro-organisms (La Ragione *et al.,* 2002).The *inv* A gene encodes a protein in the inner membrane of bacteria, which is necessary for invasion of host's epithelial cells (Darwin and Miller ,1999). The *inv* A gene is widely used as a target in PCR assay for *Salmonella* detection (Malorny *et al.,* 2003). Several reports confirmed the successful detection of 100% of *Salmonella* species isolates from poultry using specific primers for the invA gene with no false positive or negatives (Oliveira *et al.,* 2003; Moussa *et al.,* 2010 and Ammar *et al.,* 2016). While, for the intracellular survival of *Salmonella* is regulated by the (*mgt*C) genes (Zou *et al.,* 2012), while *hilA* is a transcriptional activator essential for the regulation of the invasion process, all the *Salmonella* tested were positive for both *inv*A and *hil*A genes, show the virulence potential of the strains in relation to their ability to be invasive after the attachment to the intestinal epithelium (Barrilli *et al.,* 2018).

Therefore, the objectives of this study were to investigate the potential zoonotic spread of *Salmonella* species isolated from animal products and calves fecal matter and in-contact human being, identify them by serotyping, in addition, using PCR to confirm the results.

METHODOLOGY

Sample collection

A total of 352 samples were collected from (urban and rural areas) in Meonfiya Governorate, Egypt between January 2017 and December 2018. Overall, a total of 114 from poultry giblets (52) liver, (32) gizzard and (30) heart, (58) minced meat and (50) calves fecal matter samples were collected as aseptically as possible, they were dipped in buffered peptone water (BPW) (Oxoid Ltd.,UK) and transported to the laboratory of Department of Animal Hygiene and Zoonoses, Faculty of Veterinary Medicine, Sadat University , Egypt.

Human stool samples were collected from Shebin El-kom Fever Hospital, Shebin El- Kom Educational Hospital, Menouf fever hospital, and Central Hospital of Qweisna. For 130 stool samples collected, history on the health status [clinical history of fever, abdominal pain, vomition, diarrhea, headache and any previous medical history) and behavioural history (consumption of undercooked meat or chicken and since when it has been consumed, contact with animals or poultry, type of consumed water (source, treated / filtered or not)] and personal information [occupation, age and residence place (rural or urban)]. The stool samples were handled the same way as described for poultry giblets, minced meat and calves fecal matter samples.

Isolation and identification of *Salmonella* species

After being incubated at 37ºC over-night (18 -24 hours), one milliliter (1ml) of homogenized (BPW) was transferred to 10ml of Rappaport – Vassiliadis Soy (RVS) BROTH and incubated at 41.2°C over-night (18 -24 hours). From the enriched broth, a loopful was streaked onto the

surface of Xylose Lysine Desoxycholate (XLD, Oxoid, CM0469) agar and *Salmonella – Shigella* (S–S, lab m, LAB052) agar and the plates were incubated at 37 °C for 24 hours. The suspected purified colonies are red with black centers on XLD . While, they appeared as colorless, with or without black center, on S-S agar media, typical *Salmonella* colonies are colorless or very light pink, opaque or semi-transparent. Some of *Salmonellas* make colonies have black center.

Selection and characterization of *Salmonella* species isolates

The suspected positive results were used for biochemical identification **(**Triple Sugar Iron agar test, Oxidase test, Indol test, Methyle red test, Voges proskauer test, Citrate utilization test, Urea hydrolysis test, Sugar fermentation test and H2S production). But the most significant tests were TSI [(red slant/yellow butt), gas (+ or -) and H_2S (+ or-)] and urease (–ve).

Serotyping of *Salmonella* species isolates

Typing of *Salmonella* isolates was performed in Animal Health Research Institute, Dokki, Giza. Diagnostic monovalent, polyvalent I, II, III and monovalent *Salmonella* O and H (phase 1 and phase 2) antisera. (Denka Seiken co., LTD, Tokyo,Japan) and (Pro–lab diagnostic, U.K). The positive *Salmonella* colonies biochemically were confirmed by omnivalent antisera by slide agglutination test. Detection of *inv*A in *Salmonella* species isolates was performed using PCR as described by (Oliveira *et al.,* 2003 and Malorny *et al.,* 2003). Detection of *mgt*C in *Salmonella* species isolates was performed using PCR.

Genomic DNA Extraction

According to the G-spin^{TM} and QIAamp DNA Mini Kit, (Catalouge no.51304).

PCR Amplification

The primer used for PCR amplification is displayed in [Table 1]. PCR was performed in thermo cycler model [SENSO Quest, Germany]. The PCR mix (25µL) for each sample consisted the following: 4 µL extracted DNA, 12.5 µL Master Mix, 1 µL from forward primer, 1 µL from reverse primer and 6.5 µL ultra-pure deionized water. The amplification was done using Conditions which had been described in **(Table 6),** Expected fragments providing visible bands of appropriate size of 284bp (*inv*A) were considered positive, 655bp were considered positive for *mgt*C and 150 bp were considered positive for *hil*A.

Table 1: primer used for PCR amplification

Descreption	Target gene	Primer sequence	Amplicon size	Reference
Salmonella spp. Specific	*inv*A	F-GTGAAATTATCGCCACGTTCGGGCAA R-TCATCGCACCGTCAAAGGAACC	284bp	oliveira *et al*.,(2003).
Virulence associated gene	*mgt*C	F-TGACTATCAATGCTCCAGTGAAT R-ATTTACTGGCCGCTTGCTGTTG	677bp	Soto *et al.*, (2006).
	*hil*A	F-CATGGC TGG TCA GTTGGAG R-CGTAATTCATCGCCTAAACG	150bp	Mizusaki *et al*., (2008)

Table 6: Cycling conditions of the different PCR reactions

Gene	Primary denaturation	Secondary denaturation	Annealing	Extention	No. of cycles	Final extention
*inv*A	94ºC 5 min	94º C 30 sec.	55 º C 45 sec.	72 º C 30 sec.	35	72 º C 10 min.
*Mgt*C	94ºC 5min	94 º C 30 sec.	58 º C 45 sec.	72 º C 45 sec.	35	72 º C 10 min.
Hila	94ºC 5min	94º C 30 sec.	60ºC 45 sec.	72 ºC 30 sec.	35	72 º C 10 min.

Statistical analysis:

Data were collected, tabulated, and statistically analyzed with SPSS (Statistical Package for Social Science) version 20 by

using the Chi- Square analysis test (X^2) as was performed by (Olalekan *et al.*, 2018) to compare between two qualitative variables, it tests the significance of difference between frequencies of different observations (Peat and Barton, 2005).

$X^2 = \Sigma$ (observed – expected) ^{2} / expected

This test was used as test of significance at:

- P-value > 0.05 was considered statistically insignificant.
- P-value ≤ 0.05 was considered statistically significant.
- P-value < 0.01 was considered statistically highly significant.

RESULTS

Prevalence of *Salmonella* species in animal and human being

Table 2: Frequency distribution of *Salmonella* species isolation in animal and human samples

Source	Type sample	No. of samples	No. (%) positive for *Salmonella* spp. on XLD
Poultry giblets	Liver	52	37(71.15)
	Gizzard	32	22(68.75)
	Heart	30	19(63.33)
Meat	Minced meat	58	21(36.21)
Calves	Fecal matter	50	3(6)
	Sub –total	222	102 (45.9)
Human	Stool	130	55 (42.3)
	Grand –total	352	157(44.6)

Table 3: Molecular detection of *Salmonella* spp. *inv*A gene and virulence genes (*mgt*C and *hil*A) from different samples

Source	Type of animal sample and human	No. of Tested samples	No.(%) of molecularly confirmed *Salmonellae* by *inv*A (Positive)	No.(%) of molecularly confirmed by *mgt*C (Positive)	No.(%) of molecularly confirmed by *hil*A (Positive)
Poultry giblets	Liver	5	5 (5/5)	5(5/5)	4(4/5)
	Gizzard	5	5 (5/5)	3(3/5)	4(4/5)
	Heart	4	4 (4/4)	4(4/4)	4(4/4)
Meat	Frozen Minced meat	6	6 (6/6)	5(5/6)	5(5/6)
Calves	Fecal matter	2	2 (2/2)	2(2/2)	2(2/2)
Human	Stool	11	11 (11/11)	9(9/11)	11(11/11)
	total	33	33(100)	28(84.85)	30(90.9)

Table 4: the distribution of genes in the examined 33 *Salmonella* spp. isolates from different samples

No. of sample	Source	*inv*A	*mgt*C	*hil*A
1	Calf fecal matter 1	+	+	+
2	Human stool 1	+	+	+
3	Gizzard 1	+	+	+
4	Frozen minced meat 1	+	+	+
5	Human stool 2	+	+	+
6	Gizzard 2	+	+	+
7	Frozen minced meat 2	+	+	+
8	Frozen minced meat 3	+	+	+
9	Human stool 3	+	+	+
10	Heart 1	+	+	+
11	Gizzard 3	+	+	+
12	Liver 1	+	+	+
13	Human stool 4	+	+	+
14	Human stool 5	+	+	+
15	Liver 2	+	+	+
16	Liver 3	+	+	+
17	Heart 2	+	+	+
18	Gizzard 4	+	-	+
19	Liver 4	+	+	+
20	Frozen minced meat 4	+	+	+
21	Calf fecal matter 2	+	+	+
22	Human stool 6	+	+	+
23	Human stool 7	+	+	+
24	Human stool 8	+	+	+
25	Human stool 9	+	+	+
26	Heart 3	+	+	+
27	Human stool 10	+	-	+
28	Heart 4	+	+	+
29	Frozen minced meat 5	+	+	+
30	Frozen minced meat 6	+	-	-
31	Human stool 11	+	-	+
32	Liver 5	+	+	-
33	Gizzard 5	+	-	-
Total		**33(100%)**	**28(84.85%)**	**30(90.9%)**

Serogroups of *Salmonella* species isolates:

The predominant serogroups of *Salmonella* species were *S.typhimurium* , *S.enteritidis* and *S.kentucky* as in **(Table 5).**

Source	**Type of animal and human sample**	**Isolated serotype**
Poultry giblets	Liver	*S*.Typhimurium
	Gizzard	*S*.Enteritidis
	Heart	*S*.Typhimurium
Meat	Minced meat	*S*.Typhimurium
Calves	Fecal matter	*S*.Typhimurium
Human	Stool	*S*.Enteritidis (3 isolates) *S*.Kentucky (2 isolates)

Frequency of detection of invasive gene (*inv*A) in *Salmonella* species isolates

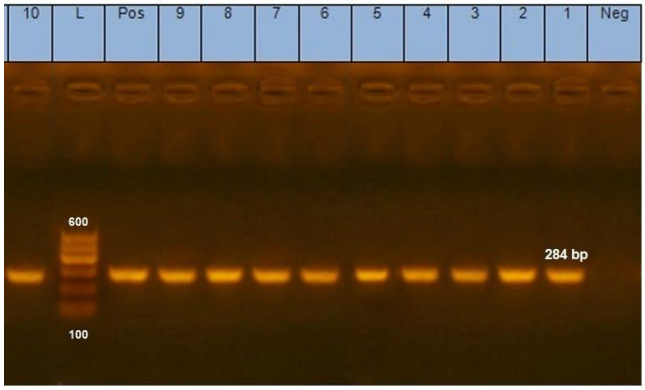
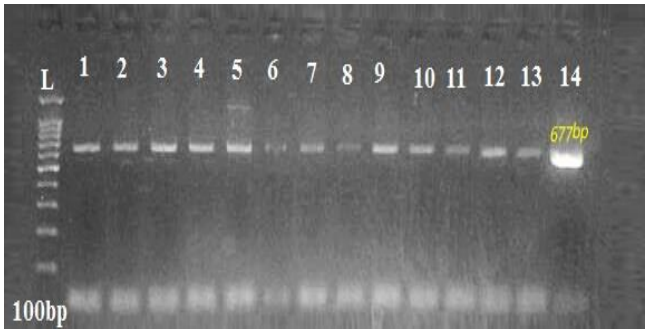


Fig (1) : the figure shows the *inv*A (284 bp) in Ethidium bromide-stained gel electrophoresis as they were performed for 10 samples which were previously serotyped (1.5 % agarose gel).

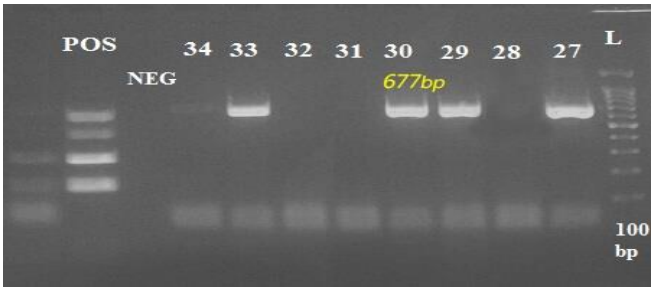
As**:**[lane 1] 10 – human stool sample 1, [lane 2] L- Gel Pilot 100 bp ladder, [lane 3] control positive provided by Animal Health research Inistitute, Egypt ,[lane 4] 9- human stool sample 2 , [lane 5] 8- human stool sample 3,[lane 6] 7- human stool sample 4,[lane 7] 6- human stool sample 5,[lane 8] 5-animal fecal matter sample,[lane 9] 4- minced meat sample,[lane 10] 3- poultry liver sample ,[lane 11] 2- poultry heart sample , [lane 12] 1- poultry gizzard sample , [lane 13] negative control.

Fig(2) Frequency of detection on *mgt*C gene in *Salmonella* species isolates



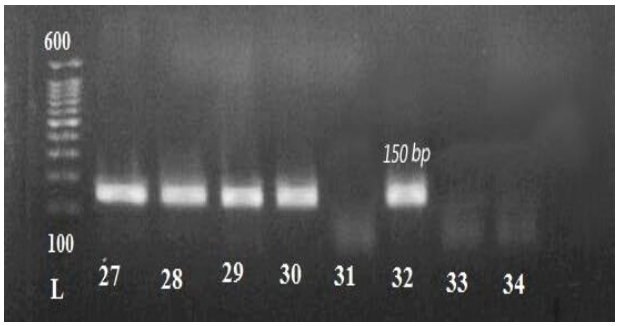
The figure shows the amplified *mgtC* (677 bp) of *Salmonella* isolates from different sources in Ethidium bromide-stained gel electrophoresis as they were performed for 14 samples (1.5 % agarose gel). As:[lane 1] L – Gel Pilot 100 bp ladder, from [lane 2] till[lane15] positive samples for *mgt*C gene.

Fig (3) Frequency of detection on *mgt*C gene in *Salmonella* spp. isolates



The figure shows the amplified *mgtC* (677 bp) of *Salmonella* isolates from different sources in Ethidium bromide-stained gel electrophoresis as they were performed for 14 samples (1.5 % agarose gel). as: direction from right to left [lane 1] L – Gel Pilot 100 bp ladder, [lane 2] positive samples for *mgt*C gene. While [lane3] negative sample for *mgt*C , [lane4]and[lane5] positive samples for *mgt*C gene , [lane6] and[lane7] negative sample for *mgt*C, [lane 8]positive sample for *mgt*C gene, [lane9]negative sample for *mgt*C,[lane10] gene,control negative and [lane11] control positive.

[figure4] Frequency of detection on *hil*A gene in *Salmonella* spp. isolates



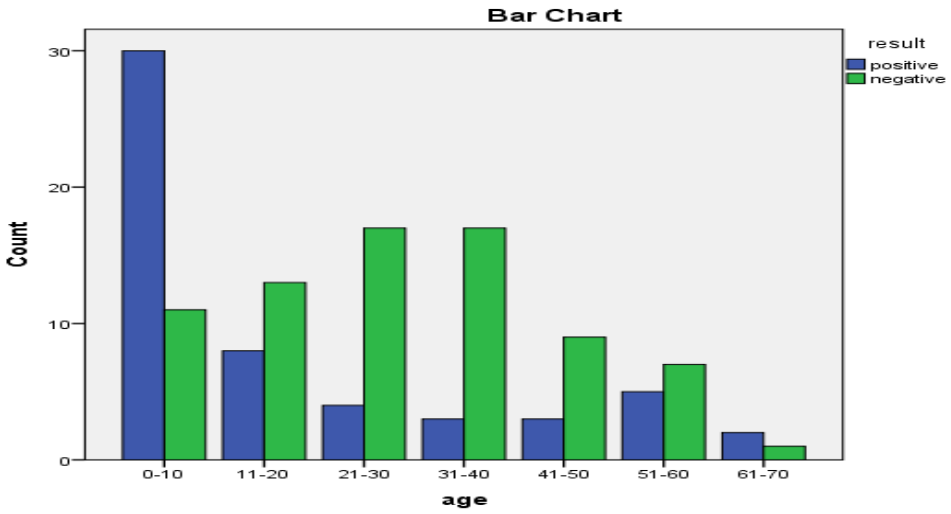
The figure shows the amplified *hil*A(150 bp) of *Salmonella* isolates from different sources in Ethidium bromide-stained gel electrophoresis as they were performed for 14 samples (1.5 % agarose gel). as:[lane1] L – Gel Pilot 100 bp ladder , from [lane2]till[lane5] positive for *hil*A gene , [lane6] negative for *hil*A gene ,[lane7] positive for *hil*A gene and [lane8 and lane 9] negative for *hil*A gene.

Table 6: The association between age of patients and *Salmonella* infection according to result of bacteriological and biochemical examination of stool samples

		Result		Total
		positive	Negative	
Age	0-10	30	11	41
	11-20	8	13	21
	21-30	4	17	21
	31-40	3	17	20
	41-50	3	9	12
	51-60	5	7	12
	61-70	2	1	3
Total		55	75	130
Chi- square		**29.122****		
P- value		**0.000058**		

** highly statistically significant.

Fig. (5): showing the association between age of patients and *Salmonella* spp. infection according to bacteriological and biochemical examination of stool samples.



DISCUSSION

The study was conducted using the 'One Health' concept which encircles the interaction of animals, humans and the environment in the transmission of diseases (CDC, 2017). The primary goal was to study the public health implications of salmonellosis in animals and human in Menofiya Governorate, Egypt, inhabiting the same area. The annual report of Rapid Alert System for Food and Feed reported that *Salmonella* species is the most notified food-borne pathogen with 70% of infections related to poultry (RASF, 2010).
Chicken meat and products were linked with increased incidence of Salmonellosis in many developing countries including India, Egypt, Brazil and Zimbabwe (Yang *et al.*, 2011). Contamination of poultry meat and products with *Salmonella* species can occur during production, processing, distribution, retail marketing, handling and preparation.

In this study, bacteriological and biochemical examination of 352 samples detected 157 (44.6%) *Salmonella* species isolates, including 71.15% (37/52), 59.4% (19/32), 60% (18/30), 24.14% (14/58), 6 %(3/50) and 42.3% (55/130) in liver, gizzard, heart, frozen minced meat, calves fecal matter and human stool, respectively, as shown in (table 2). The obtained result was in agreement with Menzies *et al.*, (1994) who isolated *Salmonella* from avian sources with an incidence of 34.5%, Waltman et al., (1992) studied the prevalence of *Salmonella* in spent laying hens. *Salmonella* was isolated from 2418 of 3700 (65.4%) caecal pools, Cardinale et al., (2003) isolated *Salmonella* from 96 (32%) of 300 samples processed and Bada-Alambedji et al., (2006) examined 120 chicken carcasses for the presence of *Salmonella* which isolated from 75 (62.5%) of the examined samples.

Among several authors who detected higher isolation rates (Ahmed *et al*., 2008) reported 64.3% in Bangladesh, (Ramya *et al.*, 2012) reported 50% in India and (Rahimi, 2012 and Sodagari *et al.*, 2015) reported slightly lower rates of 18% and 21.6% in Iran. While in Egypt higher rate of 40% was reported by (Abd El-Aziz, 2013) and (Abd El-ghany *et al.*, 2015) who detected 32% of contaminated chicken organs. In addition, respective higher isolation rates of 28, 29, 29.3, 38, 64, 44, 35, 32.5 and 46.7% were previously obtained from poultry meat and organs in Maryland and Illinois (Maung *et al.*, 2006), Iran (Sodagari *et al.*, 2015), India (Ruban *et al.*, 2010), Pakistan (Soomro *et al.*, 2010), India (Ramya *et al.*, 2012), Egypt (Abd El-Aziz , 2013), Lahore City (Ahmed *et al.*, 2013) and Brazil by (Das Chagas *et al.*, 2013 and De Oliveira *et al.*, 2014).

The higher isolation rate of *Salmonellae which* was reported from liver and heart samples could be attributed to the contamination of these organs from the crop and the intestinal contents during evisceration (Abd El-ghany *et al.*, 2015).

On the contrary, lower isolation rates were reported by (Hossain et al ., 2006) who detected *Salmonella* species with an incidence of (11.42%), (Roy *et al.*, 2002) who isolated 569 *Salmonella* out of 4745 (11.99%) from poultry, poultry product, (Norberg, 1981) who found that the percent of isolation of *Salmonella* reached (11.5%) and (Dahal, 2007) who analyzed 400 samples of chicken carcasses to detect the prevalence of *Salmonella* in them and found that 13% were positive for *Salmonella*.

Moreover, lower rates of 6, 4 and 5% were previously reported in India (Anumolu and Lakkineni, 2012), Iraq (Nader *et al.*, 2015) and Egypt (Tarabees *et al.*, 2017), respectively. Furthermore, slightly higher percentage of 10.6 and 11.8 were reported by (Kozacinski *et al.*, 2006) in Croatia and (Kaushik *et al.*, 2014) in India, respectively.

The prevalence in this study was high as shown in (table 2), which could be attributed to increase level of contamination of product from the retailer and by naked eye inspection some of samples have pathogenic lesions such as liver samples had multifocal sub-capsular necrosis in the visceral and parietal surfaces while some of heart samples had necrotic foci on the myocardium. As a result the difference in the prevalence rates between various studies could be attributed diversity in sampling methods, season and isolation techniques (Sodagari *et al.*,

2015), in addition to the sanitation and hygienic measures during transportation, slaughtering and de-feathering of carcasses (Rahimi, 2012 and Sodagari *et al.,* 2015). In China for example, 28.3% of retail chicken meat samples were contaminated with *Salmonellae*, this percentage was higher than reported in abattoirs indicating the poor hygienic measures in markets (Li *et al.,* 2013).

In this study the isolation rate from frozen minced meat was 36.21% as in (table 2), which was higher than that obtained by (Hussein, 2019) who examined a total of 312 chicken and beef samples including their processed products for the presence of *Salmonella* species, the isolation rate was 16.5%, 20%, 13.5% and 10% in raw meat, minced meat, sausage and beef burger, respectively with an overall percentage of 15%. On the other hand, our result was lower than that reported by (Van *et al.,* 2007) who revealed the presence of *Salmonella* in retail chicken and beef samples as (53.3% and 62%).

The difference in isolation rates may be due to The presence of *Salmonella* species in minced meat, meat preparations and meat products is related to the origin of meat used in production (epizootic situation, primary production, slaughter line, cutting, cold storage, hygiene practice of employees) (Rašeta *et al.,* 2017).

It was no surprise that the isolation rate of *Salmonella* in this study from calf fecal matter was (6%) as in (table 2), which was nearly similar to that reported by (Haggag and Khaliel, 2002) (4%), and (Younis *et al.,* 2009) (4.09%). On contrary, this result was much lower than that reported by (Moussa *et al.,* 2010) (43.53%) from diarrheic calves and (27.69%) from apparently healthy contact calves.

Differences of the prevalence rates of *Salmonella* in diarrheic calves in comparison to the previous studies could be explained in the light of species and geographical locations and hygienic measures, and these factors significantly influence the prevalence of salmonellosis in calves (Younis *et al.,* 2009).

Concerning human stool samples, the isolation rate was 42.3% which was nearly similar to the isolation rate obtained by (Delarocque *et al.,* 1998) who detected 59% of children in France and lower than that obtained in in Norway (87.2%) as described by (Kapperud *et al.,* 1998), and slightly higher than that obtained in Thailand, Egypt and India (18% , 23.5 % 14.3%) as described by (Bodhidatta *et al.,* 2002; Hamed , 2005 and Suresh *et al.,* 2004).

While, lower isolation rates of 7.2, 6.2 and 10% were reported in Nigeria (Agada *et al.,* 2014), Ethiopia (Beyene and Tasew, 2014), Iraq (Nader *et al.,* 2015), respectively. Furthermore, in Egypt, the prevalence of *Salmonellae* in stool samples were reported as 6% (El-bahnassi , 2005), 10% (Rabie *et al.,* 2012), 6% (Ahmed *et al.,* 2014) and 4% (Gharieb *et al.,* 2015).

In this study, the prevalence of having salmonellosis due to food intake is higher than contact with animal. By stool culturing it is 33.08% (43/130) for dealing with animal and 62.30% (81/130) due to food intake. This matching the fact that salmonellosis occurrence is due to fecal-oral route (Murray *et al.,* 2007).

Young children, older adults, and people with weakened immune systems are the most likely to have severe infections as shown in (Table 6), The disease can affect all species of domestic animals but young animals and pregnant and lactating animals are the most susceptible (OIE , 2018 and WHO, 2018).

(Table 5), shows that the identified *Salmonella* serovars of serotyping 10 samples which were carried out by Animal Health research Inistitute, Egypt. The most prominent serovars were *S.*Typhimurium (40%), *S.*Enteritidis (40%) and *S.*Kentucky (20%) and this was in correspondence with Vose *et al.,* (2013), who reported that *Salmonella enterica* serovar *Entritidis* and *Typhimurium* were the most predominant isolated organisms in most cases associated with the consumption of contaminated poultry products and this also was in agreement with the Centers for Disease Control and Prevention (CDC, 2009). In addition, the predominant serovars present in Egyptian poultry farms were *S.*Typhimurium and *S.*Enteritidis (Abd El-Ghany *et al.,* 2012 and El-Sharkawy *et al.,* 2017).

In contrary, other study in Egypt reported that *S*.Enteritidis predominated and followed by *S*.Typhimurium in samples of chicken origin (Abd –Elghany *et al.,* 2015). While in human stool samples, only *S*.Enteritidis and *S*.Kentucky were detected by serotyping and this was in agreement with the fact that Gastroenteritis is the principle manifestation of *Salmonella* species infection. However, invasive infections have been reported due to *S*.Enteritidis more than *S*.Typhimurium in recent years (Ispahani and slack , 2000).

S.Kentucky currently ranks among top ten serovars causing gastroenteritis in humans (Bonalli *et al.,* 2012). While the poultry source of this serovar has been also previously reported (Boyle *et al.,* 2010).

In this study, the relationship between obtaining *Salmonella* infection and age of patient was statistically significant, as shown in (Table 6). The increased susceptibility to have *Salmonella* infection is higher in young age than adults and elders having severe infection may be due to weak immune system and more contact with the surrounding environment and this was in agreement with WHO (2019).

In this study, PCR assay was carried out for the detection of the *inv*A gene from 33 isolated strains after being identified bacteriologically and biochemically (10 of them were subjected for further serological confirmation). Our results, revealed that the gene was present in all of the isolates(100%) as demonstrated by the presence of a 284 bp PCR amplified fragment (fig.,1) which was in agreement with the previous studies (Dias *et al.*,2003 ; Mir *et al.,* 2010 ; Dione *et al.*,2011 and Ali 2017). Furthermore, PCR on the same 33 isolates detected (84.85%) for *mgt*C and (90.9%) for *hil*A gene.

*Inv*A is a putative inner membrane component of SPI-1, essential for entry into epithelial cells, and it is a specific target gene for confirmation of *Salmonella* spp. , while *hil*A is a transcriptional activator essential for the regulation of the invasion process , All the *Salmonella* tested were positive for both *inv*A and *hil*A genes, show the virulence potential of the strains in relation to their ability to be invasive after the attachment to the intestinal epithelium (Barrilli *et al.,* 2018) and this coincides with the result in this study as *inv*A was detected as 100% while *hil*A was 90.9% as in (table3) (fig.,1) and (fig.,4). This was as similar as all the isolates were positive for at least five virulence-related-genes (*inv*A, *hil*A, *stn*, *ssr*A, *sip*C). The same phenomenon has been highlighted in *S. typhimurium* isolated in swine, where all the strains analysed were positive for *sip*A, *sip*D, flgK, flgL,fljB, *inv*A, *sop*B and *sop*E2 genes (Barrilli *et al.,* 2018).

The *hil*A gene is important for the expression of the type III secretion components required for invasion of host epithelial cells and induction of apoptosis in macrophages (Bajaj *et al.,* 1996). Consistent with the results of the current study, *hil*A gene was previously identified in 100 and 88.2% of *Salmonellae* isolated from chicken samples in Brazil (Borges *et al*., 2013) and Egypt (Ammar *et al*., 2016), respectively, while, in Zambia, none of *Salmonella* species isolates harbored *hil*A gene (Ulaya, 2013), while the gene was identified in 8.3% (Gharieb *et al.,* 2015) and 8.6% (Akbarmher, 2010) of *Salmonella* spp. isolates.

The *mgt*C 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 (Günzel *et al.,* 2006) and (Chai et al., 2012). The result of *mgt*C gene in this study was 84.85% as in (table3), (fig.,2) and (fig., 3), lower than the result of (Ahmed *et al.,* 2016) who detected it in 100% of *Salmonella* species isolates.

While decreased ability to survive within human monocytic cells observed with a *S.typhi* SPI (*Salmonella* Pathogenicity Island) -3 strain could be overcome with an *mgtC*-containing plasmid, which restored the wild type phenotype at 2 and 24 hours post-infection. This means that *mgt*C is a virulence factor playing a major role that is not supplied by any other bacterial factor codified either inside SPI-3 or in the entire chromosome of *Salmonella* and is also present in the chromosome of other *Salmonella* serovars (Retamal *et al.,* 2009).

Various virulence determinants in *Salmonella* spp. are associated with chromosomal and plasmid factors (oliveria et al., 2003). These factors are encoded by several genes. Most of the genes required for *Salmonella* virulence are clustered within five pathogenicity islands(SPI_S) which contribute to its success as an intracellular pathogen (Hensel, 2004). The SPI encode a type III secretion system (TTSS), which inoculates bacterial effector proteins through bacterial and host membranes to interact with host cells (Marcus *et al.,* 2000).

According to (Table 4), the considerable differences in virulence determinants of *Salmonella* serovars in this study may be attributed to the variation in sample sources, types of serovars and presence or absence of plasmids carrying virulence associated genes, this is in accordance with (Porwollik *et al.,* 2004) and this is could be showed by (Sotohy *et al.,* 2018), who collected (95) samples from three dairy farms at 3 different localities in Assiut Province including for detection of *Salmonella* species by molecular characterization for the presence of 6 virulence genes; *pef*A (700 bp), *mgt*C (677 bp), stn (617 bp), *sop*B (517 bp), *inv*A (284 bp) and *avr*A (422 bp) in *Salmonella* isolates revealed that all 6 tested virulence genes were detected in *Salmonella enterica* serotype isolated from manure.

CONCLUSSION

poultry meat and poultry giblets (liver, gizzard and heart) contribute huge source of infection by *Salmonella* species followed by frozen minced meat. Also in coming in contact with animals constitute a risk factor. *Salmonella* Enteritidis and *Salmonella* Typhimurium were the most isolated strains from animal and human samples. Obtaining infection with salmonellosis was recorded higher due to food-intake than dealing with animals which may be attributed to the vaccination programs in farms. Besides, poultry meat, poultry giblets and meat are popular foods due to their high nutritive value and their availability on wide scales in markets.

Ethical Approval

All procedures performed in this study including collection of human fecal samples and animals were in accordance with the Egyptian ethical standards of the national research committee. All human subjects gave their consent for the collection of the fecal samples, with the agreement that any identifying details of the individuals should not be published.

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