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Detection of virulence genes and antimicrobial susceptibility of salmonella spp isolated from diarrheal human in wasit province, Iraq

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***Abstract*---**This study aimed to isolate Salmonella species from diarrheal human and to determine the antimicrobial susceptibility and virulence-associated genes. A total of 145 human stool samples were from Wasit province, during the period from November 2018 to August 2019. The isolates were identified according to colony morphology, Gram stain, biochemical, Analytical profile index strip (Api-20E) and serotyping. The antimicrobial susceptibility test was done by utilizing the VITEK-2 Compact system and a disc diffusion method. Salmonella isolates were tested to detect six virulence genes, namely sefA, mgtC, sopB, spvR, Stn and invA by PCR technique. Results showed that Salmonella spp was isolated from 9 out of 145(6.2%), S. Typhimurium 55.55%, S. Typhi 33.33%, and S. Enteritidis 11%. The highest isolation rate of species was in the group aged between 3 to 15 years (10.34%). The gender distribution was 7.14% in males and 4.91% in females. August and March recorded the highest level of isolated Salmonella which was 11.76% and 10%, respectively. Multidrug Antibiotic Resistance Index (MARI) of S. Typhimurium, S. Enteritidis, and S. Typhi were 0.4-0.86, 0.57, and 0.62-0.71, respectively. The genes of mgtC, Stn, and invA were present in all Salmonella serovars 100%, sopB, and spvR genes were present in 80%, while the sefA gene was derived in 66.67%.

***Keywords*---**salmonella, antimicrobial resistance, MDR, sopB, spvR, sefA.

Introduction

Salmonella enterica subspecies enterica are the major causes of zoonotic foodborne diseases causing a thousand deaths in human worldwide (Odoch et al., 2017; Velasquez et al., 2018; Eguale, 2018). Non-typhoidal *Salmonella* (NTS) infections have shown an increasing incidence, there are approximately 94 million cases of NTS gastroenteritis resulting in 155,000 deaths in the world each year and 80.3 million cases were estimated as a foodborne origin, *Salmonella* Typhimurium, *Salmonella* Enteritidis, *Salmonella* Heidelberg, and *Salmonella* Newport are the epidemiologically important NTS serovars as they are the majority of human *Salmonellosis* globally ((Niki *et al.*, 2017;Eguale, 2018; Li *et al.*, 2018; Adhikari *et al.*, 2018). Multidrug resistance (MDR) bacteria are currently considered an emergent global disease and a major public health problem((Roca *et al.*, 2015). The antimicrobial resistant *Salmonella* in both human and animal infections is concerned a problem in the world, especially in developing countries where the risk of infection is growing due to unhygienic living status. In addition, antimicrobial resistance and MDR among *Salmonella* serovars especially, NTS because of the widespread use of antimicrobial as therapeutic or prophylactic medication in both human and animal treatments, besides, the use of antimicrobial drugs as growth stimulators in animal production ((Feasey et al., 2012; WHO, 2012; Abraham et al.,2014).*Salmonella* possesses many virulence factors associated with pathogenicity and the severity of infection depending on the existence of the virulence genes such as invasion A (*invA*), which assists the bacteria to invade the host cell and the virulence gene called enterotoxin (*stn*), which encodes a protein causing acute diarrhea ((Malorny et al., 2003; Moore and Feist, 2007; Zou et al., 2012). The current study aimed to isolate *Salmonella* spp from diarrheal human and detect the associated virulence genes and antimicrobial susceptibility in Wasit province.

Materials and Methods

Isolation of *Salmonella*

One hundred and forty five stool samples were collected (1. 5-5 gm) from patients suffering from diarrhea, nausea, vomiting, and abdominal pain that were suspected infected with *Salmonella* from the Al-Zahra teaching hospital and Al-Karama teaching hospital from November 2018 to August 2019. The samples were taken into a sterile container containing 15 ml of Cary-Blair (Pronadisa /Spain) and transported to the microbiology laboratory. About one gm stool from Cary-Blair medium using a disposable sterile spatula and transferred into buffered peptone water (Oxoid /UK) as pre-enrichment step and incubated at 37°C for 24 hours, then one ml of the pre-enrichment broth inoculated to nine ml of Tetrathionate broth (Himedia /India) for enrichment step, incubate at 41°C for 48hours, after that a loopful from the enrichment broth streak onto salmonella –shigella agar (Biomarklabs /India) and *Xylose Lysine Deoxycholate* (Himedia /India)) and incubated at 37°C for 18-24 hours. The suspected colonies (pale colonies on salmonella -shigella agar or red colonies on *Xylose Lysine Deoxycholate* with or without black centers were picked up and subcultured onto Brilliance Salmonella Agar Base and then incubated at 37°C for 24 hours ((WHO, 2010 b; Ezat et al., 2016). The purified colonies were subcultivation on nutrient

agar slants (Oxoid/UK) and incubated for identification on level *Salmonella* spp by API 20E (Bio-merieux /France) and serotyping was done at the Institute of Public Health, Baghdad, Iraq.

Antimicrobial Susceptibility, MDR, and multiple antibiotics resistance (MAR) index detections

Salmonella isolates were tested for their susceptibility to eight antimicrobials drugs using Automated system VITEK-2 Compact system which to depend on Minimum Inhibitory Concentration (MIC) which includes Amikacin 30mg, Cefepime 30mg, Ceftazidime 10 mg, Ciprofloxacin 5 mg, Gentamicin 10 mg, Imipenem 10 mg, Piperacillin/Tazobactam 30-6 mg, Trimethoprim/Sulfamethoxazole 1. 25–23. 75 mg. While in vitro, thirteen different antimicrobials which are Ampicillin 25mg, Amoxicillin20mg \Clavulanic Acid 10mg , Ampicillin10mg \Sulbactam 10mg, Aztreonam 30mg , Cefixime 5mg , Cefoxitin 30mg , Clarithromycin 15mg , Doxycycline 30mg , Erythromycine 15mg , Methicillin 5mg , Metronidazole 30mg , Oxacillin 5mg , Tetracycline 30mg using antimicrobials disc Susceptibility test was assayed according to the (EUCAST, 2019). MDR was detected according to Magiorakos et al. (Magiorakos et al.,2012), the isolates are considered as MDR when resistant to three or more separate antimicrobial classes. MAR index was calculated by dividing (A): the number of antimicrobial drugs resistant of isolate by (B): the total number of antimicrobial drugs, where the same isolate which exposed, the results more than 0.2 was considered high risk (Krumperman,1983).

Molecular identification of certain virulence genes (*invA*, *sefA*, *mgtC*, *sopB*, *spvR* and *stn*)

Genomic DNA of five *Salmonella* serovars isolates was extracted according to the protocol of ABIO pure Extraction. Conventional polymerase chain reaction (PCR) was performed with six sets of primer which are *invA*, *sefA*, *mgtC*, *sopB*, *spvR* and *stn* as shown in (Table1). PCR mixtures (20µl) consisted of Master Mix 10 µL; Forward primer and Reverse primer 1µl to each; Nuclease Free Water 6µl and 2µl of the template (DNA). PCR amplifications were performed in a thermal cycler(BioRad /USA)), PCR programs performed as follows: initial denaturation 95ºC for 5 min and1cyle; denaturation at 95ºC for 30 sec and 30 cycles; annealing at 60 ºC for *mgtC* and *sopB*, 55 ºC for *sefA* and *stn* and 63 ºC for *invA* and *spvR* for 30 sec and 30 cycles; extension at 72ºC for 1 min and 30 cycles; final extension at 72ºC for 7 min and 1, cycle and hold at 4 for 10 min and 1 cycle. The products of PCR were separated by electrophoresis in a 1% agarose gel and stained with ethidium bromide and the fragments were transilluminated with UV light.

Table1
Primers sequence used in this study

Gene	Primer sequence (5' to 3')	Product size/bp	reference
sefA	F\ 5`-GATACTGCTGAACGTAGAAGG-3` R\ 5`-GCGTAAATCAGCATCTGCAGTAGC-3`	488	Oliveira *et al.* 2002

mgtC	F\ 5`-TGACTATCAATGCTCCAGTGAAT-3` R\ 5`-ATTTACTGGCCGCTATGCTGTTG-3`	655	Soto *et al.*,2006
sopB	F\ 5`-GATGTGATTAATGAAGAAATGCC-3` R\ 5`-GCAAACCATAAAAACTACACTCA-3`	1170	
spvR	F\ 5`-CAG GTT CCT TCA GTA TCG CA-3` R\ 5`-TTT GGC CGG AAA TGG TCA GT-3`	310	Pasmans *et al.*,2003
stn	F\ 5`-CTT TGG TCG TAA AAT AAG GCG-3` R\ 5`-TGC CCA AAG CAG AGA GAT TC-3`	260	Makino *et al.*,1999
invA	F\ 5`-GTG AAA TTA TCG CCA CGT TCG GGC AA-3` R\ 5`-TCA TCG CAC CGT CAA AGG AAC C-3`	284	Kumar *et al.*,2008

Results

Isolation of *Salmonella* from diarrheal human

Salmonella serovars isolates were 9 out of 145 (6.2%), all isolates were belonging to S. enterica subsp. enterica I. NTS was the most isolated 6/9 (66.66%) and 3/9(33.33%) were TS which was *S.*Typhi. *S.* Typhimurium serovar was the most common 5/9(55.55%) followed by *S.* Enteritidis 1/9(11%). Non-significant difference at P <0.05 between age groups .The highest isolation rate 6/58(10.34%) of *Salmonella* serovars were isolated from the age group 3-15 years old followed by the age group of 16-62 years old 3/63(8.3%), there were no *Salmonella* isolates detected in infants 0/51(0%) aged between one month to two years . Males recorded a high rate of infection 7.14% compared with females 4.91% with Non-significant difference at P <0.05 between male and female (Table 2).

Table 2
Distribution of Salmonella serovars according to gender

Gender	NO	Positive result	%	OR (95% CI)	X2 , P-value
Male	84	6	7.14	1.487 (0.357-6.196)	0.300, 0.584(NS)
Female	61	3	4.91		
Total	145	9	6.2%		

Antimicrobial susceptibility, MDR and Multidrug Antibiotic Resistance Index (MARI) of *Salmonella* serovars

The antimicrobial susceptibility testing revealed absolute resistance 100% of NTS and TS to Methicillin, Oxacillin, Amoxicillin \Clavulanic Acid, Cefoxitin, Metronidazole, Erythromycin and Clarithromycin. S. Typhi was resistant 100% to Aztreonam, Amikacin and Gentamicin and 66.66% to Ceftazidime, Tetracycline, and Doxycycline. In addition, S. Typhimurium showed 100% resistance to Cefixime, 75% to Ampicillin \Sulbactam and 50% to Ceftazidime and Cefepime. While S. Enteritidis was 100% resistant to Ampicillin \Sulbactam, Aztreonam, Amikacin, Gentamicin and Doxycycline (Table 3). None of the *Salmonella* serovars were sensitive 100% to all antimicrobial agents tested. The isolates were resistant to nine and more antimicrobial and the prevalence of MDR was 100%. The MARI

of S. Typhimurium was ranging from 0. 4-0. 86, S. Enteritidis 0. 57 and S. Typhi 0. 62-0. 71(Table 4).

Table 3
Antimicrobial susceptibility testing of isolated *Salmonella* serovars

Antimicrobial	Nontyphoidal						Typhoidal		
	*S.*Typhimurium (4)			*S.*Enteritidis(1)			*S.* Typhi (3)		
	S %	I %	R %	S %	I %	R %	S %	I %	R %
Methicillin	0	0	100	0	0	100	0	0	100
Cefoxitin	0	0	100	0	0	100	0	0	100
Metronidazole	0	0	100	0	0	100	0	0	100
Erythromycin	0	0	100	0	0	100	0	0	100
Clarithromycin	0	0	100	0	0	100	0	0	100
Oxacillin	0	0	100	0	0	100	0	0	100
Amoxicillin \Clavulanic Acid	0	0	100	0	0	100	0	0	100
Cefixime	0	0	100	0	0	100	0	0	100
Tetracycline	25	50	25	100	0	0	0	33.33	66.66
Aztreonam	25	0	75	0	0	100	0	0	100
Ampicillin \Sulbactam	25	0	75	100	0	0	66.66	0	33.33
Doxycycline	50	25	25	0	0	100	33.33	0	66.66
Ampicillin	75	0	25	100	0	0	0	66.66	33.33
Amikacin	25	0	75	0	0	100	0	0	100
Gentamicin	25	0	75	0	0	100	0	0	100
Ceftazidime	50	0	50	100	0	0	33.33	0	66.66
Cefepime	50	0	50	100	0	0	33.33	0	66.66
Ciprofloxacin	75	0	25	100	0	66.66	0	0	33.33
Piperacillin/Tazobactam	75	0	25	100	0	0	100	0	0
Trimethoprim/Sulfamethoxazole	75	0	25	100	0	0	100	0	0
Imipenem	75	0	25	100	0	0	100	0	0

Table 4
Multidrug antibiotic resistance index (MARI) isolated Salmonella spp in human

Salmonella Spp	*S.*Typhimurium	*S.*Typhimurium	*S.*Typhimurium	*S.*Typhimurium	*S.*Enteritidis	*S.* Typhi	*S.* Typhi	*S.* Typhi
No.AR*	18	16	9	12	12	14	15	13
MARI	0.86	0.76	0.4	0.57	0.57	0.66	0.71	0.62

* No.antibiotic resistance

Molecular identification of virulence-associated genes among Salmonella serovars

sefA gene was present in 66. 67% of testing three isolates, it was found in S. Enteritidis and S. Typhi, and not present in *S.* Typhimurium. *mgtC*, *stn* and *invA* genes were detected in all of the tested serovars 100%. While *sopB* and *spvR* genes were present in 80% of isolates, (Table5 and Fig 2,3 and 4).

Table 5
Distribution of virulence genes among different *Salmonella* serovars

No. of isolate	serovars	virulence genes					
		sefA	*mgtC*	*sopB*	*spvR*	*stn*	*invA*
113	*S.* Enteritidis	+	+	+	+	+	+
II	*S.*Typhimurium	ND	+	-	+	+	+
III	*S.*Typhimurium	ND	+	+	+	+	+
IV	*S.*Typhimurium	-	+	+	+	+	+
S115	*S.*Typhi	+	+	+	-	+	+
Total %		2	5	4	4	5	5
		66.67%	100%	80%	80%	100%	100%
X2, P-value		4.306 , 0.506					

NS: Non-significant difference at P <0.05, ND: not done

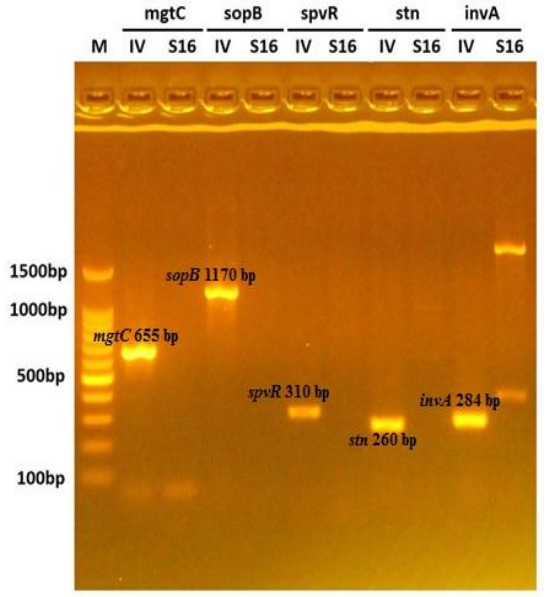

Figure 1: Results of the presence of *mgtC*, *sopB*, *spvR*, *Stn* and *invA* gene of IV human Salmonella isolate: showed positive amplification. DNA bands were separated by electrophoresis on a 1% agarose gel (at 100v/mAmp for 75 min). Ethidium bromide stained gel agarose bands were visualized using a gel imaging system under UV transilluminated. Lane1:100bp DNA ladder. * S16 isolate was ignored because it did not give any results with these *Salmonella* genes.

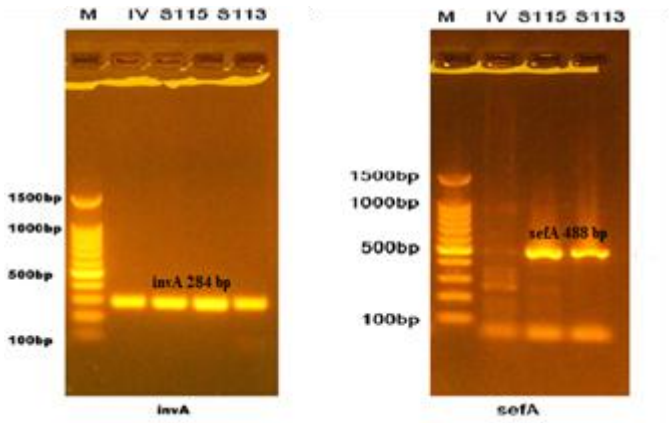

Figure 2: Results of the presence of invA and SefA gene of IV, S115 and S113 human Salmonella isolates were fractionated on 1% agarose gel electrophoresis stained with Ethidium bromide. Lane1:100bp DNA marker (at 100v/mAmp for 75 min). gel agarose bands were visualized using a gel imaging system under UV transilluminated.

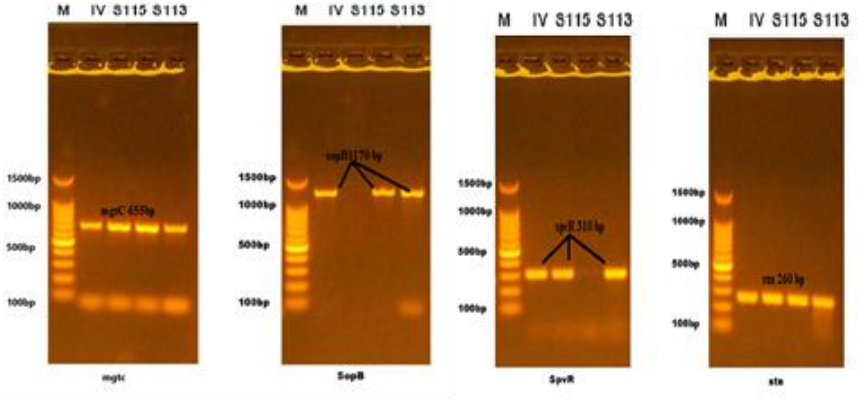

Figure 3: Results of the presence of *mgtC* and *sopB, Spvr* and *Stn* gene of IV,s115 and s113 human *Salmonella* isolates were fractionated on 1% agarose gel electrophoresis stained with Ethidium bromide.Lane1:100bp DNA marker (at 100v/mAmp for 75 min). Ethidium bromide stained gel agarose bands were visualized using a gel imaging system under UV transilliminated.

Discussion

The prevalence of *Salmonella* serovars were 6.2% and the most common isolates were NTS, S. Typhimurium serovar was a high prevalence followed by S. Typhi and *S.* Enteritidis. All the serovars belonged to the *Salmonella* enterica I subspecies which is responsible for most of the human Salmonellosis (Brenner *et al.*,2000). The percentage of NTS in this study was lower compared to findings in Iraq 15%, 10.3%, 14.8% respectively (Al-Rajab et al., 1988;Harb et al., 2017 ;Harb et al., 2019). And in a study conducted in Karbala, Iraq, it was found that S. Typhimurium serovar was detected 12.7% lower than our finding (Alaa and Al-

Abbas, 2018). In the world, *Salmonella* spp were found at 20.8%, 39% and 7.3% (Mas¨ud and Tijjani, 2015; Siourimè *et al.* ,2017; Gebreegziabher *et al.*, 2018) respectively. On the other hand, the prevalence of *Salmonella* in the present study was higher than in other studies, *Salmonella* was isolated from 4.7% of bloody diarrhea in children and 0.5% from human in in Baghdad and Al-Diwaniya, Iraq (Al-Kubaisy et al., 2015;Jawad and Al-Charrakh, 2016). In the world, a study conducted in Turkey, *Salmonella* has isolated at 5.2%(Küçük et al., 2016)..*S.* Typhimurium serovar was the high prevalence which agreed with the findings of (Harb et al. ,2017; Harb et al. 2019) who reported that S. Typhimurium was the predominant serovar among Iraqi children with a prevalence of 54.5% and 62.2%, respectively. Similarly, other studies found that *S.* Typhimurium was the predominant serovar in human at 28.4%, 10%, and 23% ((Ravel et al., 2010;Ahmed et al., 2016;Andoh et al., 2017). *S.* Typhi was found at 33.33% and S. Enteritidis at 11% which were in line with other studies that reported S. Typhi at 11.71% and S. Enteritidis at 7.69% in human (Andoh et al., 2017). In addition, *S.* Typhi isolated 21%, 6.2 %, 19.5% from diarrheal human reported by(Siourimè et al., 2017; Muhammed et al., 2018; Gebreegziabher et al., 2018). While in human endemic Salmonellosis found *S.*Enteritidis the predominant serovar at 20.8% (Ravel et al., 2010). Another study found *S.* Typhi was a higher prevalence serovar at 25.8% followed by S. Typhimurium at 23.5% and *S.* Enteritidis at 19.9% (Mas¨ud and Tijjani, 2015).

Although there was no significant difference between age groups, the highest infections were detected among 3-15 years age group followed by age group 16-32 years, whereas not isolated from infants one month to two years this partially accordance with other studies, children under 5 years reported 10.3% (Alaa and Al-Abbas, 2018), and children aged between one and two years found significantly higher odds of Salmonellosis 21%, 73.7% respectively compared with those 9 years old at 5.3% (Gebreegziabher *et al.*,2018). Nonisolated *Salmonella* from infants between one month –two years in this study may be due to that they are entirely dependent on breastfeeding, in addition to the use of hygienic methods by mothers during natural or artificial milk feeding of their infants. A study investigates that exclusive breastfeeding for the first six months of age is protective against enteric diseases among infants such as *Salmonella* infections((Williams et al., 2016). Children had a higher rate of *Salmonella* isolates in the current study, this is because children have a habit of putting dirty fingers or soiled objects in their mouths, playing in soil contaminated with feces, and are less likely to wash their hands after defecation compared to adults, in addition, adults do not usually predispose themselves to various contaminated areas than youth (Mashi,2013;Gebreegziabher *et al.*,2018). Infections in males were higher without significant difference, this result was in agreement with Harb and co-author who reported higher *Salmonella* infection in males 56.3% than that in females 43.7%(Harb *et al.*,2017), Lamboro et al. (2016) found that males infection was higher 52.63% than females 47.37%, and with Gebreegziabher et al. (2018) when detected *Salmonella* in males higher 63.2% than females 36.8% with no significant difference. These results may be attributed to that females, due to social and religious tradition, are less probably to eating, drink outdoor, and using hygienic measures than males. Moreover, females may be less risky to occupational risks of farming, contaminated food and drink, contaminated environment and activities (Abdullahi et al., 2010).

i*nvA*, *stn* and *mgtC* genes were detected in all *Salmonella* serovars, spvR and sopB genes were present at 80% of isolates, while spvR was non detected in S. Typhi serovar and *sefA* gene was found only in *S.* Enteritidis and S. Typhi. These results agree with other studies, *invA* and *sopB* found 100% in *S.* Enteritidis and S. Typhimurium, *stn* presence 100% in S. Typhimurium and 90% in S. Enteritidis (Borges et al.,2019). *S.* Typhimurium isolates from human and chicken meat were positive 96.7% for the *sopB* gene (Ahmed et al.,2016). In contrast, *sopB* gene present in S. Typhimurium at rate of 50%, in S. Enteritidis at 66.66% and *stn* gene in S. Typhimurium at 50% and in S. Enteritidis at 100% (Thung *et al.*,2017). In another study, *invA* found 100% in S. Typhimurium and S. Enteritidis isolates, *mgtC* found 37.5% in S. Enteritidis, 44.44% in S. Typhimurium while, *stn* found 33.33% and 75% in S. Typhimurium and S. Enteritidis respectively (Elkenany *et al.*,2019). In a study, S. Typhimurium isolated from milk and dairy products were possessed both *invA* and *stn, mgtC*, *sopB*, *spvR,* and *invA* found 100% in S. Enteritidis (El-Baz *et al.*, 2017; Omar *et al.,* 2018; Jassim and Al-Gburi,2020). The *sefA* gene was present only in S. Typhi and S. Enteritidis but not in S. Typhimurium in the present study, which agreed with a study that found that S. Typhimurium isolated from milk was luke the *sefA* gene(Jassim and Al-Gburi,2020). This gene was found in specific serovars including S. Enteritidis, S. Gallinarum, S. Pullorum, S. Dublin, S. Rostock, S. Seremban, and S. Typhi, in addition, this gene was used as a specific, and practical method for direct diagnosis of *S.* Enteritidis and *S.* Gallinarum in chickens (Gong et al., 2016; Borges et al., 2019). This gene detected 100% in S. Typhimurium and S. Enteritidis (Chaudhary et al., 2015). spvR was not found in S. Typhi in this study, the *spv* locus is strongly associated with strains that cause nontyphoid bacteremia, but are not present in typhoid strains, indicating that the pathogenesis and immunology of typhoid have fundamental differences from the syndrome of non-typhoid bacteremia (Guiney and Fierer, 2011). The presence of 100% *invA* and *stn* genes and other virulence genes indicated that the *Salmonella* isolates were highly invasive, enterotoxigenic, and high pathogenic and implying that these strains have the necessary virulence gene capable of playing an important role in causing severe infection, and the spread of Salmonella *invA* and *stn* virulence genes may be utilized as a gene marker for the fast recognition of the virulent strains of *Salmonella* (Elgohary et al., 2017).

High resistance TS and NTS to Methicillin, Oxacillin, Amoxicillin \Clavulanic Acid, Cefoxitin, Metronidazole, Erythromycin and Clarithromycin, high resistance to the second generation cepalosporines cefoxitin and third generation cepalosporines cefixime, both S. Typhimurium and S. Typhi resistance to fourth-generation cefepim and third generation cepalosporines ceftazidime. In addition, S. Typhimurium and S. Typhi serovars are resistant to Ampicillin \Sulbactam, ciprofloxacin and Tetracycline, while S. Typhi was sensitive to piperacillin/Tazobactam and Imipenem counter to S. Typhimurium. These results agree fully or partially with the results of others, NTS isolated from children found resistance to ciprofloxacin 57%, trimethoprim\sulfamethoxazole 51.5%, gentamicin 27.3%, tetracycline 78.8% and amoxicillin/ clavulanate 6.1% (Harb et al., 2017). S. Enteritidis and S. Typhimurium from human stool sample found resistance respectively to tetracycline (59.3%, 33.33%) ciprofloxacin, (27.7%,

15.15%), gentamicin (3.7%, 3%), cefotaxime (0%, (6%), amoxicillin /clavulanic acid (12. 9%, 33.33%), cefoxitin (1.9%, 6%), ceftazidime (3.7%, 6%) reported by (Andoh et al., 2017). TS and NTS isolated from human found resistance against tetracycline 89.5%, gentamicin 15.8%, and all were sensitive to ciprofloxacin (Gebreegziabher et al.,2013). S. Typhimurium isolated from chicken meat and human was highest resistance against trimethoprim /sulfamethoxazole (73.3%, each), tetracycline 53.3%, gentamicin 30%, and 2.7% of the isolates were resistant to cefotaxime and ceftriaxone each (Ahmed *et al.*,2016). S. Typhi isolated from diarrheal human were found resistant to amoxicillin/clavulanic acid 42%, trimethoprim/sulfamexazol 33%, tetracycline 50%, gentamicin, cefotaxime, imipenem, and ciprofloxacin (0%), while other studies found S.Typhi resistant 100% for tetracycline and erythromycin (Siourimè et al.,2017; Njum *et al.*,2019) .

Cephalosporins are the drugs of choice for children because they cannot be treated with fluoroquinolones. Salmonella exhibiting resistance to cephalosporins is an emergent problem worldwide (WHO, 2012). Unfortunately, report an alarmingly high prevalence of MDR among *Salmonella* serovars and high risk, MARI value higher than 0. 2 which indicates high risk (Miranda et al., 2009). S. Typhi strains isolated from human infected with typhoid fever in Iraq showed an MDR of 46.15% (Aljanaby and Medhat, 2017). NTS isolated from children had an MDR of 84.9% 28. The increasing occurrence of *Salmonella* serovars resistant to sulfonamides, βlactam, and aminoglycosides is considered alarming, as they are used for the treatment of invasive Salmonellosis and subsequently, multi-drug resistant Salmonellae constituted a public health hazard and potentially affected the efficacy of medications in humans (Lekshmi et al., 2017). The alarming resistance profiles, high MDR, and high risk of *Salmonella* in this study might contribute to the fact that in Iraq, as in many other developing countries, After the Iraq war in 2003, there has been little central governance on antibiotic use in humans or the animal sector and antimicrobial drugs are directly purchased from pharmacies without medical prescription(Jassim,2010). And the use of antimicrobials in animal husbandry and the resultant development of antibiotic resistance in microorganisms is recognized as a potential human health concern because the antibiotic-resistant bacteria associated with animals may be developed pathogenic to humans and transmitted of antibiotic-resistant bacteria to humans via food from farm animals (Chang et al.,2015).

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