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Molecular Identification and Phylogenetic Analysis of *Salmonella* species Isolated from Diarrheal Children and Dogs in Baghdad Governorate, Iraq

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A B S T R A C T

This work aimed to use conventional PCR to identify *Salmonella* spp. that were isolated from diarrheal children and healthy and diarrheic dogs based on four virulence genes, *hilA*, *stn*, *spvR*, and *marT*. Sixteen *Salmonella* isolates including: 9 isolated from children's diarrhea from three species (*S.* Typhimurium, *S.* Enteritidis, *S.* Typhi) and seven isolated from dogs including (*S.* Typhimurium, *S.* Enteritidis, *S.* Muenchen), were identified primarily by several methods. The PCR products of the *16S rRNA* gene were sequenced and examined using BLAST analysis to find differences and similarities between these Iraqi isolates and already-known global strains in order to construct the phylogenetic tree of *S.* Muenchen which was detected for the first time in dogs in Iraq. The results of the study revealed that all isolates of *Salmonella* obtained from children possess the *hilA* and *stn* genes. The *marT* gene was detected in 88% of the *Salmonella* serovars, and the *spvR* gene was carried in 55% of the isolates. Among dog *Salmonella* isolates, the *hilA* gene was detected at 100%, the *stn* gene was at 85.7%, the *marT* gene was present at 71.4%, while the *spvR* gene was found at 57.1%. The result of DNA sequencing and phylogenetic tree indicated that the local Iraqi *S.* Muenchen was extremely close to the national strain and share the same *16S rRNA* gene sequence, the isolate was registered at NCBI and became a global reference with the accession number OQ999043.1. In conclusion, the presence of these important virulence genes among *Salmonella* serovars isolated from children and dogs alerted on the potential risk of contamination of the environment and may lead to a community health crisis.

Keywords: *hilA* gene, *marT* gene, *Salmonella* Muenchen, phylogenetic tree, PCR, children, dog, Iraq

INTRODUCTION

Salmonella is one of the zoonotic-borne pathogens, that can infect human and various animals' species, it causes serious public health threats and economic consequences due to the associated healthcare costs in the world (1, 2). Three clinical syndromes caused by *Salmonella* include typhoid, enteritis and bacteremia, nontyphoidal *Salmonella* (NTS) is the most common causative agent of diarrhea and gastroenteritis in children, and the most prominent serovars are *S.* Typhimurium and *S.* Enteritidis, the mortality rate may reach1.87 million yearly in under five years children, especially in developing countries (3, 4). This pathogen was transmitted by ingestion of contaminated food and water or by contact with animals (5-7). Dogs are considered as a potential source of zoonotic *Salmonella* transmission, they have been reported to harbor and shed *Salmonella* in their feces sub clinically, and it was found that the most frequent *Salmonella* serovars isolated from humans corresponded with the more prevalent *Salmonella* serovars in dogs. Canine Salmonellosis is characterized by diarrhea, fever, abdominal pain, vomiting and lethargy (8-10). S. Muenchen is another serovar of *Salmonella* that has been isolated from

humans, animals, including dogs, and food (11-13). This serovar can cause acute salmonellosis, including fever, abdominal cramps, diarrhea, vomiting, in addition to the complications of S. Muenchen that may extend beyond classic gastrointestinal symptoms by causing rhabdomyolysis and myocarditis (14, 15).

Virulence factors of *Salmonella* include *Salmonella* pathogenic islands (SPIs), toxins, flagella, fimbriae and plasmids related to virulence. These virulence factors do not present in all *Salmonella* serovars; thus the pathogenicity and virulence of *Salmonella* serovars are associated with the absence and presence of virulence factors, and numerous studies have focused on the study of various *Salmonella* virulence factors (16-18).

The chromosomally encoded *Salmonella* enterotoxin (*stn*) gene is playing a main role in *Salmonella* virulence via the maintenance of *Salmonella* membrane composition and integrity, and it is toxic to intestinal epithelial cells leading to intestinal disorders and diarrhea, this gene is prevalent in all *Salmonella* serotypes and contains sequences unique making it an ideal PCR target for the diagnosis of *Salmonella* strains (19, 20). Hyper-invasive locus (*hilA*) gene is one member of the family of transcription regulators which has a potential role in the regulation and production of effector proteins of T3SS in SP-1 which are expressed by regulators and assist *Salmonella* in colonization and invasion of the intestinal epithelium (16, 21). The membrane-associated regulator (*marT*) gene is located on SPI-3 and encodes a regulator protein responsible for regulating the expression of the misL gene, also other genes that are regulated by *marT* expressed proteins essential in *Salmonella* pathogenicity such as biofilm regulators, fimbriae formation, in addition to antigenic surface proteins (22, 23). *Salmonella* plasmid virulence R (*spvR*) gene is a regulator gene of the spvABCD genes. Although *spvR* is present in most non typhoidal *Salmonella* and absent in Typhoidal *Salmonella* (TS), it was reported in S. Typhi isolates (24, 25). The spvABCD plays a role in the multiplication of *Salmonella* intracellularly, and survival of *Salmonella* within macrophages, also potentiate the systemic spread of pathogen (26, 27).

Molecular phylogenetics has emerged as a crucial technique for genome comparisons, it is employed to categories metagenomic sequences and identify genes, and the phylogenetic trees for different *Salmonella* serovars have been studied in many investigations (28, 30). Thus, this study was conducted to detect the *hilA*, *stn*, *marT*, and *spvR* virulence genes in *Salmonella* serovars isolated from diarrheal children and dogs and to perform phylogenetic analysis depending on the *16Sr RNA* gene of the Iraqi S. Muenchen isolate, which was isolated from a dog for the first time in Iraq, to determine the differences and similarities compared to existing global strains.

MATERIALS AND METHODS

Ethical Approval

The experimental design and procedures carried out in this study were reviewed and approved in accordance with animal welfare ethical standards by the Research Ethics Committee at the University of Baghdad's College of Veterinary Medicine with ethics number 999/P.G. dated on Jun 26, 2022.

Sample Collection

150 stool samples were collected from children suffering from diarrhea, were less than 12 year of age and 165 fecal samples from dogs including 90 apparently healthy dogs and 75 diarrheic dogs in Baghdad governorate, Iraq during the period between September/2022 to March/2023.

Sample Processing

Salmonella was isolated and identified from diarrheic children stool sample and dog's fecal sample has been confirmed by VITEK 2 compact system, API20E and serotyping test in previous study (58). The isolates were cultured in 10 milliliter of Brain heart infusion broth medium and incubated at 37 °C overnight in shacking incubator (31). Further confirmation was done by Polymerase Chain Reaction (PCR) and analysis of DNA sequencing.

Extraction of Genomic DNA

The genomic DNA purification Kit, manufactured by Macrogen, Korea was used to extract DNA of the *Salmonella* isolates.

Primers and PCR Reaction

The primers used in the present study are listed in Table 1, four primers were used to detect the virulence genes of *Salmonella* serovars which are *hilA*, *stn*, *marT* and *spvR* genes, in addition, the partial *16S rRNA* was used for *S.* Muenchen. The PCR reaction mixture (25 µL) contained Taq PCR Pre Mix 5 µL, forward and revers primer 1 µL to each, DNA template 1.5 µL, and distilled water 16.5 µL. Then, the thermo cycler (Promega, USA) started the program as follows: initial denaturation: one cycle, at 95 °C for 3 minutes; denaturation: 35 cycles, at 95 °C for 35 sec except for *16S rRNA* 45 sec; annealing: 35 cycles, at 55 °C for 30 sec for *hilA*, *stn*, *marT* and *spvR* genes ant 56 °C for 45 sec; extension: 35 cycles, at 72 °C for 45 sec except for *16S rRNA* one min; and final extension: 1 cycle; at 72 °C for 7 min for the 4 genes and 5 minutes for *16S rRNA* gene.

DNA Sequencing, Phylogenetic Analysis and Tree Construction

The PCR product of the S. Muenchen was sent to Macrogen Corporation/ Korea for getting the sequencing of the target *16S rRNA* gene. Sequence data were then aligned

by BLAST (Basic Local Alignment Search Tool) at the National Centre for Biotechnology Information database (NCBI) to search for a similar sequence previously recorded in the NCBI, and the phylogenetic tree was constructed using the maximum composite likelihood and minimum evolution method by Molecular Evolutionary Genetics Analysis (MEGA) version 6.0. software which eliminated all positions containing missing data and gaps (32)

Table 1. Sequence of primers used this study

Gene		Primer Sequence (5" to 3")	GC%	Product size bp	Reference
hilA	F	CTGCCGCAGTGTTAAGGATA	50	496	33
	R	CTGTCGCCTTAATCGCATGT	50		
stn	F	CTTTGGTCGTAAAATAAGGCG	43	260	34
	R	TGCCCAAAGCAGAGAGATTC	50		
marT	F	CGTCGTCTCACAACAAACATTC	45	556	16
	R	CTGACAAATCAATGCCGTAACC	45		
spvR	F	CCGCTGAGCAGGGTTATTT	53	723	16
	R	CTTGGTCGGGTAATACAAGGAG	50		
16S rRNA	F	AGAGTTTGATCCTGGCTCAG	50	1250	35
	R	GGTTACCTTGTTACGACTT	42.1		

RESULTS

Isolation and Identification

Salmonella isolates, nine isolates of *Salmonella* serovars were identified from children suffering from diarrhea, including three isolates of each *S*. Typhimurium, *S*. Enteritidis and *S*. Typhi, and seven isolates from dogs, including three isolates of both the *S*. Typhimurium and *S*. Enteritidis and one isolate of *S*. Muenchen used in the molecular study.

Detection of Virulence Genes

The DNA fragments successfully amplified with a length of 496 bp were evaluated as positive for the *hilA* gene (Figure 1). The bands of approximately 260 bp represented partial amplification of the *stn* gene (Figure 2), 556 bp bands represented partial amplification of the *marT* gene (Figure 3) and the bands of approximately 723 bp represented partial amplification of the *spvR* gene showed amplification of 723 bp DNA fragments (Figure 4).

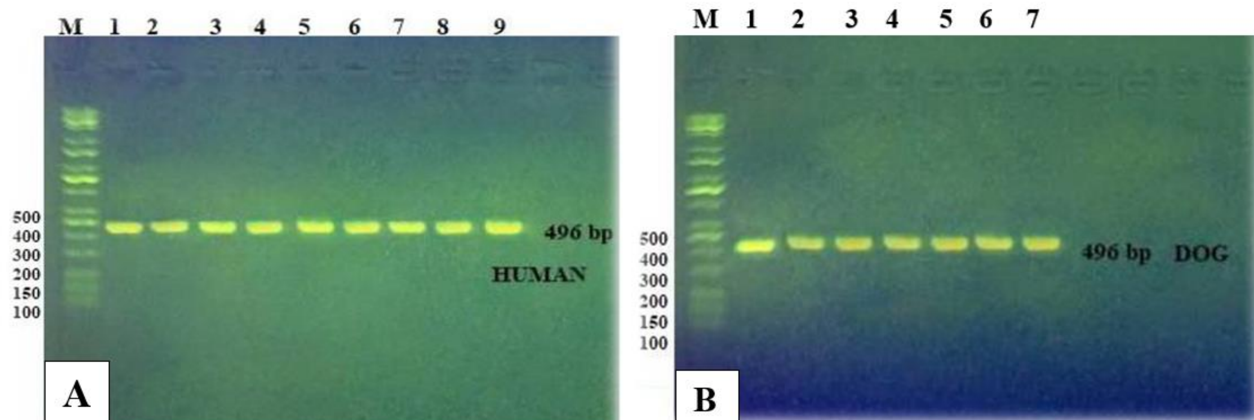

Figure 1. (A) The PCR reaction showing *Salmonella* serovars positive for *hilA* gene in band size (496 bp). Children isolates: lanes (1-3) represent *S*. Typhimurium, *S*. Enteritidis (4-6) and (7-9) S. Typhi (7-9). **(B)** Dogs' isolates: Lanes (1-3) represent *S*. Typhimurium, *S*. Enteritidis (4-6) and *S*. Muenchen (7). The product was electrophoresis on (1.5 %) agarose. 1x TAE buffer for 1:30 hour. M: DNA ladder (100- 10000) 70V / 80 A.

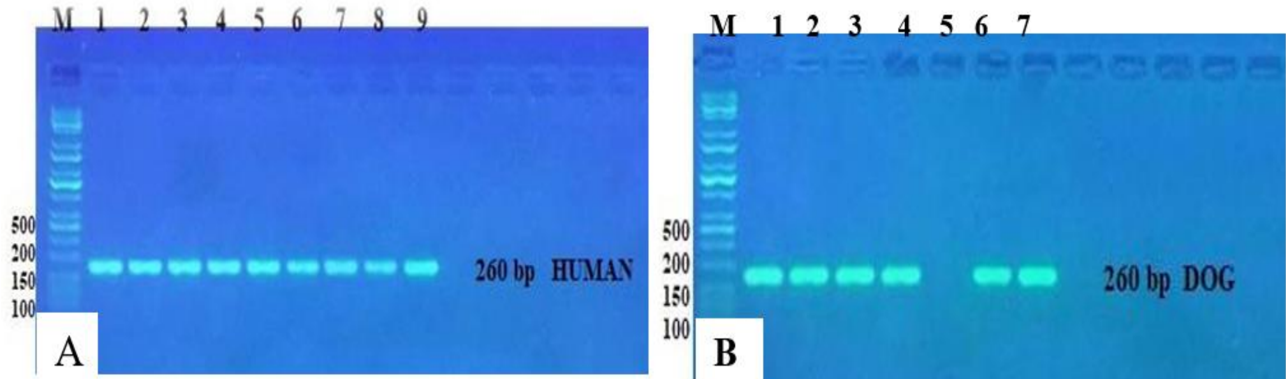

Figure 2. (A) The PCR reaction showing *Salmonella* serovars positive for (*stn*) gene in band size (260 bp). Children isolates: lanes (1-3) represent *S.* Typhimurium, *S.* Enteritidis (4-6) and (7-9) *S.* Typhi (7-9). **(B)** Dogs' isolates: lane (1-3) represent *S.* Typhimurium, *S.* Enteritidis (4-6) and *S.* Muenchen (7). The product was electrophoresis on (1.5 %) agarose. 1x TAE buffer for 1:30 hour. M: DNA ladder (100- 10000) 70V / 80 A

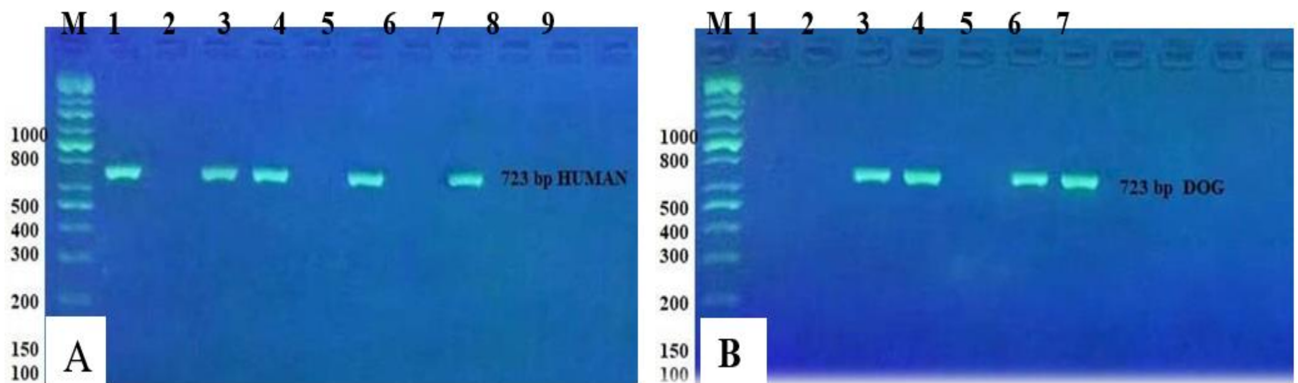

Figure 3. (A) The PCR reaction showing *Salmonella* serovars positive for (*spvR*) gene in band size (723 bp). Children isolates: lanes (1-3) represent *S.* Typhimurium, *S.* Enteritidis (4-6) and (7-9) S. Typhi (7-9). **(B)** Dogs' isolates: Lanes (1-3) represent *S.* Typhimurium, *S.* Enteritidis (4-6) and *S.* Muenchen (7). The product was electrophoresis on (1.5 %) agarose. 1x TAE buffer for 1:30 hour. M: DNA ladder (100- 10000) 70V / 80 A

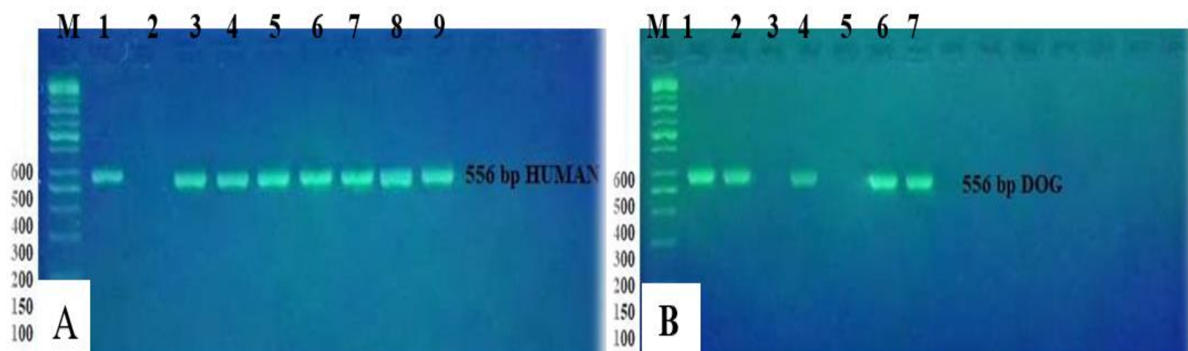

Figure 4. (A) The PCR reaction showing *Salmonella* serovars positive for (*marT*) gene in band size (556 bp). Children isolates: lanes (1-3) represent *S.* Typhimurium, *S.* Enteritidis (4-6) and (7-9) S. Typhi (7-9). **(B)** Dogs' isolates: Lanes (1-3) represent *S.* Typhimurium, *S.* Enteritidis (4-6) and S. Muenchen (7).The product was electrophoresis on (1.5 %) agarose. 1x TAE buffer for 1:30 hour. M: DNA ladder (100- 10000) 70V / 80 A.

Salmonella children isolates showed that *hilA* gene and *stn* gene were detected at 100% in all *Salmonella* serovars, 55.5% of the isolates harbored *spvR*; 66.66% in both *S.* Typhimurium and *S.* Enteritidis, and 33.33% in *S.* Typhi. While 88.88 % of the isolates harbored the *marT* gene; in *S.* Enteritidis and *S.* Typhi was present at 100%, and 66.66% in *S.* Typhimurium (Table 2).

Table 2. Presence of virulence genes in *Salmonella* serovars isolated from children

	Virulent genes, n(%)			
Serovar	*hilA*	*stn*	*spvR*	*marT*
S. Typhimurium(3)	3/3 (100)	3/3 (100)	2/3 (66.7)	2/3 (66.7)
S. Enteritidis(3)	3/3 (100)	3/3 (100)	2/3 (66.7)	3/3 (100)
S. Typhi(3)	3/3 (100)	3/3 (100)	1/3 (33.3)	3/3 (100)
Total	9	9	5	8

Salmonella dogs' isolates showed that the *hilA* gene was detected 100% in all isolates, 85.7% of the isolates carried the *stn* gene, it was present 100% in both *S.* Typhimurium and *S.* Muenchen and 66.66% in *S.* Enteritidis. The *spvR* gene was detected at 57.1% of the isolates as 100%, 66.66% and 33.33% in *S.* Muenchen, *S.* Enteritidis and *S.* Typhimurium respectively. While *marT* gene was detected at 71.4%; it was at 100% in *S.* Muenchen, and 66.66% in *S.* Typhimurium and *S.* Enteritidis (Table 3).

Table 3. Presence of virulence genes in *Salmonella* serovars isolated from dogs

	Virulent genes, n(%)			
Serovar	*hilA*	*stn*	*spvR*	*marT*
S. Typhimurium (3)	3/3 (100)	3/3 (100)	1/3 (33.3)	2/3 (66.7)
S. Enteritidis (3)	3/3 (100)	2/3 (66.7)	2/3 (66.7)	3/3 (66.7)
S. Muenchen (1)	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)
Total	7	6	4	5

Molecular Analysis of *16S rRNA* Genes

Salmonella Muenchen isolate from fecal sample of the dog were further analyzed for the presence of *16S rRNA* gene according to the response state reported in (Table 1). PCR results of *16S rRNA* gene demonstrated the presence of *16S rRNA* gene which display like a bundle on agarose gel with a size of 1250 bp show in Figure 5.

Sequencing of *16S rRNA* Gene

Salmonella Muenchen *16S rRNA* gene sequencing isolated from dog fecal sample were analyzed in NCBI GenBank database and compared with isolated sequences retrieved from the GenBank databases. The sequenced DNA were showed a match of 99% of S. Muenchen strain found in NCBI has the accession numbers (ID: CP045063.1), these matches could be distinguished by three different nucleic acid substitutions as in Table (4) illustrated that the nearest national strain possessed the Sequence ID: CP045063.1 has compatibility of 99% with three variants including; Transversion (G/T) in the location 4439251 and Transition (C/T and A/G) in the location 4438569 and 4439257, respectively. Iraqi *S.* Muenchen was registered at NCBI and established as a global reference with the accession number: OQ999043.1.

Table 4. Identical level of S. Muenchen isolate with nearest national strain in NCBI

	Source: *S.* Muenchen, *16S ribosomal* RNA gene				
No.	**Substitution**	**Site**	**Nucleotide**	**Sequence ID with compared**	**Identities**
1	Transition	4438569	C/T	CP045063.1	99%
	Transversion	4439251	G/T		
	Transition	4439257	A/G		

Phylogenetic Tree of the *16S rRNA* Gene from *S.* Muenchen

The Iraqi isolates recorded in NCBI were in concordance of 99.64% with isolates from different sources including ten USA (ID: CP045063.1, ID: CP045059.1, ID: CP082727.1, ID: CP082506.1, ID: CP051389.1, ID: CP075048.1, ID: JQ694474.1, ID: JQ694472.1, ID: JQ694469.1, ID: JQ694470.1) and Mexico (ID: CP074332.1) as shown in Table 5 and Figure 6.

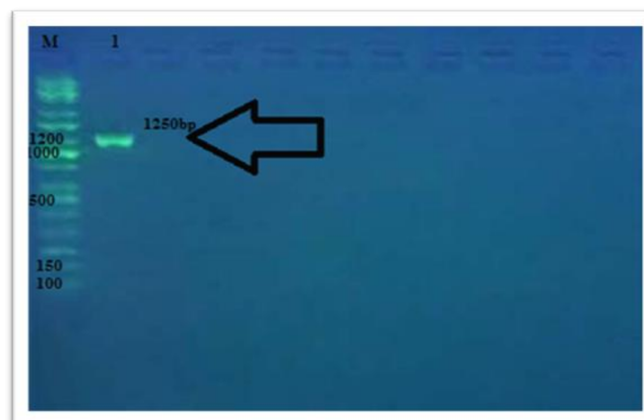

Figure 5. PCR result of the *16S rRNA* gene from *S.* Muenchen, with a band size of 1250 bp for the *16S rRNA* gene. The item was electrophoresed on 1.5% agarose in 1x TAE buffer for 1:30 h. DNA ladder: 100–10,000; 70V–80A

Table 5. Comparative analysis of the *16S rRNA* gene for *Salmonella* enterica subspecies enterica serovar *S. Muenchen* isolates

No.	Accession	Land	Source of isolate	Compatibility
1.	CP045063.1	USA, Escalon	almond drupe	99%
2.	CP045059.1	USA, CA	almond drupe	99%
3.	CP082727.1	USA, MD	ground turkey	99%
4.	CP082506.1	USA, TN	cattle-dairy cow	99%
5.	CP051389.1	USA, NC	swine	99%
6.	CP075048.1	USA, NC	water stream	99%
7.	CP074332.1	Mexico	water	99%
8.	JQ694474.1	USA, CA	swab from food production	99%
9.	JQ694472.1	USA, Irvine	food	99%
10.	JQ694469.1	USA, Fairchild	food	99%
11.	JQ694470.1	USA, Fairchild	food	99%

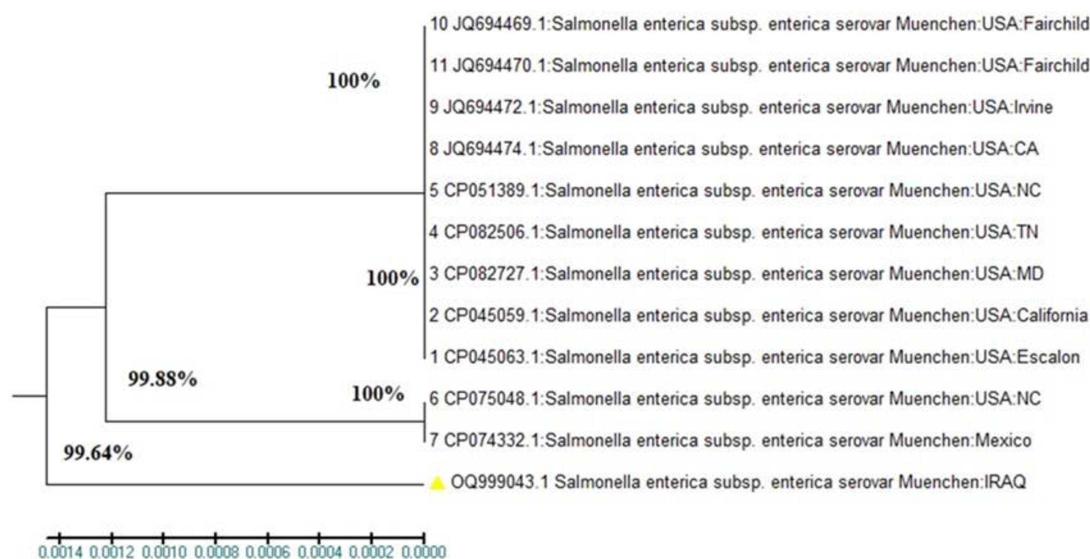

Figure 6. Phylogenetic tree with of S. Muenchen. The isolate used in the current study labeled with yellow color. MEGA software version 6.0 was used to create the analysis using the neighbor-joining method

DISCUSSION

The results of the present work indicated that the *hilA* gene was carried by all *Salmonella* serovars 100% isolated from children and dogs, this ratio corresponded with other studies around the world such as Ma et al. (2022) (36), who revealed that the prevalence rate of *hilA* gene was 100% in each of *S.* Typhimurium, *S.* Enteritidis and *S.* Typhi isolated from patients. Likewise, Lozano-Villegas et al. (2023) (37) detected *hilA* genes at 100% of *Salmonella* isolated from gastroenteritis in humans. A study recorded that *S.* Typhimurium, *S.* Enteritidis and *S.* Infantis isolated from diarrheal patients carried the *hilA* genes at 100% (38). *S.* Enteritidis from patients with gastroenteritis were positive 100% for *hilA* gene (39). In contrast, Yue et al. (2020) (16) showed that the detection rate of *hilA* gene in *S.* Typhimurium was 95.24%, and in *S.* Enteritidis isolated from diarrheal children was 83.33%. In the present study, all the dog's *Salmonella* isolates carried the *hilA* gene. There are only few studies investigated *hilA* gene in *Salmonella* isolated from dogs. A study conducted by Enany et al. (2021) (40) stated that *S.* Typhimurium in dogs was positive for the *hilA* gene 100%. A contrasting result was obtained by Ulaya, (2014) (41) who recorded the absence of *hilA* gene among 18 screened *Salmonella* isolate0% collected from dog fecal sample.

Salmonella isolates from children were positive 100% for the *Salmonella* enterotoxin gene in the present study, this result is similar with the findings reported by Maaroof and Dhayea (42) who found the incidence of the *stn* gene in the *Salmonella* isolates was 100%; Ali et al. (2019) (43) observed that the *stn* gene was found at 100% for *S.* Typhimurium and *S.* Muenchen. Moreover, the *stn* virulence gene was present 100% in *S.* Enteritidis isolated from human stool samples (44), in *S.* Typhimurium, *S.* Enteritidis and *S.* Typhi isolates from the human with diarrhea (45). In this study, the s*tn* gene was present at 85.7% of the dogs' isolates, this result is different from the finding reported by

Yildiz and Demirbilek (2023) who found dogs' *Salmonella* isolates were positive 100% for *stn* gene. However, *S.* Typhimurium isolated from dog were negative for *stn* gene as reported by (47). *S.* Muenchen isolates from patients were positive 100% for the *stn* gene (43). The *stn* gene had toxic activity and is responsible of *Salmonella* entero-toxigenic toxicity, and strongly linked with acute gastroenteritis (39, 48).

The *spvR* gene was present at 55.5 % and 57.1 % of children and dogs' isolates, respectively, 66.66% and 33.33% in *S.* Typhimurium, and 33.33%, 66.66% in *S.* Enteritidis, its detection rate was 33.33%, *S.* Typhi in children and 100% in *S.* Muenchen in dogs. The present results are similar with other findings, in which *spvR* gene was detected in 62.5% of *Salmonella* isolates, as in *S.* Enteritidis and *S.* Typhimurium isolated from human stools was 80% and 66.66% respectively (49). Frequency of *spvR* gene in *Salmonella* isolated from diarrheic human was 80%, it was 100% in *S.* Enteritidis and *S.* Typhimurium (45). A study reported that *spvR* was 75% in *S.* Enteritidis and 0% in *S.* Typhimurium isolates from human with diarrhea (16). *Salmonella* might be virulent when the *spvR* gene was absent (50). Although, the *spvR* gene was substantially correlated with *Salmonella* serovars that cause NT bacteremia, particularly *S.* Typhimurium and *S.* Enteritidis, which interprets the reason bacteria harboring this plasmid cannot cause gastroenteritis but are not present in TS serovars (24). The *spvR* gene was detected in *S.* Typhi in the present study at 33.33%, this disagrees with other studies which revealed that *S.* Typhi serovars were negative for *spvR* (45, 51, 52). In contrast, Somda et al. (2018) (25) showed that *spvR* is present at 46.15 % of *S.* Typhi isolates from diarrheic children, and suggesting that the existence of these virulence genes in *S.* Typhi from clinical samples indicates the ability of these strains to cause disease in susceptible hosts.

The data from this study showed that the *marT* gene had a high percentage 88.8% of diarrheal children *Salmonella* isolates; it's found 100% in both *S.* enteritidis and *S.* Typhi, and 33.33% of *S.* Typhimurium isolates. While 71.4% of dogs *Salmonella* isolates possessed the *marT* genes, 66.66% in *S.* Typhimurium and *S.* Enteritidis isolates and 100%in *S.* Muenchen. This result in agreement with that of Yue et al. (2020) (16) who identified the *marT* gene in 96.9% of *Salmonella* isolates belonged to *S.* Typhimurium 100% and *S.* Enteritidis 91.97% that collected from children with diarrhea. Ma et al., (2022) (36) have revealed that the detection rate of *marT* gene in *S.* Typhimurium, *S.* Enteritidis and *S.* Typhi isolates from human patient was 100%. There were no further studies on *marT* gene in human or dogs; it was detected in *Salmonella* isolated from seafood and retail chickens' sample (20, 53). The virulence gene *marT* was located in *Salmonella* pathogenicity islands-3 (SPI-3), and can be used as virulence markers for the detection of *Salmonella*, and it is involved in biofilm formation and *Salmonella* pathogenicity (16, 23). The PCR detection of *hilA*, *stn*, *marT*, *spvR* genes among the isolates of *Salmonella* may indicate the highest risk by these zoonotic bacteria into humans.

Kaabi and AL-Yassari (2019) (54) reported that the *16S rRNA* gene was sequenced most widely for *Salmonella* identification and the discovery of novel species. It was reliable markers for the phylogenetic analysis (55). The widely used of *16S rRNA* gene sequencing for *Salmonella* species identification and genetic evolutionary studies may be due to the fact that this gene was fixed and needed a very long time to change and also because the *16S rRNA* gene sequence databases were available at GenBank. This interpretation was supported by (56, 57).

Result of *16S rRNA* gene sequencing and phylogenetic data analysis showed that the Iraqi isolate of S Muenchen of fecal sample of dog was highly similar to national strains from countries far from Iraq, such as USA and Mexico with different sources revealing a new strain of *Salmonella* that was not recorded in Iraq previously. The reason for this may be due to the import of living dogs and other animals, as well as the import of meat and poultry from these countries as human and dog food, in addition to the entry of tourists from these countries to Iraq.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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الكشف الجزيئي والتحليل الوراثي لأنواع السالمونيلا المعزولة من الأطفال المصابين بالإسهال والكلاب في محافظة بغداد، العراق

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الخلاصة

يهدف هذا العمل إلى استخدام تفاعل البلمرة المتسلسل التقليدي للتعرف على السالمونيلا. التي تم عزلها من الأطفال المصابين بالإسهال والكلاب السليمة ظاهريا والمصابة بالإسهال وفقا إلى أربعة من جينات الفوعة *spvR* , *stn*, *hilA* , و*marT*. وتم تحديد ستة عشر عزلة من السالمونيلا: تسع منها معزولة من الاطفال المصابين بالاسهال ومن ثلاثة انواع (S. Typhimurium, S. Enteritidis, S. Typhi) وسبع منها معزولة من الكلاب وتتضمن (S. Typhimurium, S. Enteritidis, S. Muenchen)، وتم تحديدها بشكل اولي بعدة طرق. تم معرفة تسلسل منتجات PCR لجين *16S rRNA* وكذلك فحصها باستخدام تحليل BLAST لإيجاد الاختلافات والتشابهات بين هذه العزلات العراقية والسلالات العالمية المعروفة من أجل بناء الشجرة الوراثية لـ S. Muenchen والتي تم اكتشافها لأول مرة في الكلاب في العراق. أظهرت نتائج الدراسة أن جميع عزلات السالمونيلا التي تم الحصول عليها من الأطفال تمتلك جينات *hilA* و*stn*. كما تم اكتشاف جين *marT* في ٨٨٪ من عزلات السالمونيلا المصلية، كما تم حمل جين *spvR* في ٥٥٪ من العزلات. ومن عزلات السالمونيلا من الكلاب، تم اكتشاف جين *hilA* بنسبة ١٠٠٪، وجين *stn* بنسبة ٨٥,٧٪، وجين *marT* بنسبة ٧١,٤٪، بينما وجد جين *spvR* بنسبة ٥٧,١٪. أكدت نتيجة عملية السلسلة الحمض النووي والشجرة الوراثية إلى أن العزلة العراقية المحلية S. Muenchen كانت متطابقة بشكل كبير للغاية مع السلالة االعالمية وتشترك في نفس التسلسل الجيني ١٦S rRNA، وتم تسجيل العزلة في NCBI وأصبحت مرجعًا عالميًا برقم الانضمام (OQ٩٩٩٠٤٣,١). وفي الختام، فإن وجود جينات الفوعة المهمة بين سلالات السالمونيلا المصلية المعزولة من الأطفال والكلاب نبه الى وجود مخاطر محتملة لتلوث بيئي وقد يؤدي إلى أزمة صحية مجتمعية.

الكلمات المفاحية: تفاعل البلمرة، عزلات السالمونيلا من الكلاب، جينات الفوعة، العزلة العراقية المحلية للسالمونيلا