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Phylogeny of Multi and Extensively Drug Resistant Salmonella enterica Isolated from Salmonellosis Patients in Basra Province Iraq

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ABSTRACT

Salmonella, a bacterial genus, is responsible for salmonellosis, a global disease that can impact both humans and animals. 80 clinical specimens were collected from individuals displaying signs of salmonellosis in Basrah province. The Salmonella isolates were obtained and identified using bacterial culturing on suitable conditions and validated techniques, such as the Vitek2 system and 16S rDNA gene amplification. A study was conducted to assess the susceptibility of antibiotics using the CLSI 2022 guidelines. The findings indicated that 7 specimens of the 80 patient specimens examined, tested positive for Salmonella. Depending on the method used to sequence the 16S rDNA gene's nucleotide sequence. Just six isolates were submitted to register their sequences in the Gene Bank-NCBI in order to diagnose them at the species and serovars level, receive accession numbers, and then determine their phylogeny. Salmonella Typhi is represented by four accession numbers (LC773417.1), (LC773418.1), (LC773419.1), and (LC773420.1). Salmonella Typhimurium is denoted by a single accession number LC773421.1. One accession number LC773422.1, is listed as Salmonella enterica only. Conclusion: PCR demonstrated a notable degree of precision when it came to identifying Salmonella spp at serovars level. Salmonella sreovars exhibiting multi and extensively drug resistance were successfully isolated and identified.

1. Introduction

Salmonella is a genus comprising a group of bacteria that cause salmonellosis, a potentially fatal foodborne illness that can affect both humans and animals. It is one of the most prevalent foodborne diseases worldwide, accounting for around 93.8 million cases and 145,000 to 161,000 deaths annually. This has a substantial socioeconomic impact on a global scale [1] [2]. The regions where the majority of cases occur are sub-Saharan Africa and South/South-East Asia [3]. Salmonella infections can be

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broadly classified into two categories. The initial category is exclusive to the human host and includes typhoidal salmonellosis (enteric fever), which is caused by S. Typhi (typhoid fever), S. Paratyphi A, B, and C (paratyphoid fever). The second category of infections consists of non-typhoidal Salmonellae (NTS), which are Salmonella enterica serovars including S. Enteritidis, S. Typhimurium, and S. Choleraesuis [4]. Salmonellosis in both humans and animals is predominantly caused by S. Typhimurium and S. Enteritidis serotypes. These pathogens are transmitted to humans through infected animals, and their production has been documented [5] [6]. Antibiotics are customarily recommended for the management of extreme instances of salmonellosis. Nevertheless, the escalating mortality rate among patients infected with multidrug-resistant (MDR) strains of Salmonella enterica has generated worldwide apprehension [7] [8] [9] [10] [11]. Cases of extensively drug-resistant (XDR) typhoid have escalated [12] [13] [14] [15] [16]. In order to establish phylogenetic relationships between isolates and identify clinical and environmental isolates, 16S rDNA gene nucleic acid sequencing has been utilized for bacteria for decades. Molecular investigation is most receptive to 16S rDNA gene analysis [17]. Genomes of bacterial strains appeared to contain numerous genes with distinct functions. As an illustration, the 16S rDNA gene in bacteria is identified by its nine hypervariable regions (V1-V9), which are utilized for species-level bacterial identification due to the sequencing diversity observed in this particular area across numerous bacterial species. Additionally, numerous bacterial strain species contain conserved regions in close proximity to hypervariable regions. The conserved regions, which correspond to the quorum sensing sequences, were amplified using a universal primer [18] [19]. These regions have been employed in numerous genetic studies [20] [21] to identify and distinguish between various bacterial species. Prior to the advent of DNA sequencing technologies, systematics and taxonomy employed phylogenetic trees to ascertain the relationships both within and between species, as well as to delineate the connections between paralogous genes within a family [22]. The objective of this research endeavor was to identify MDR and XDR Salmonella enterica, ascertain the genetic relationships among Salmonella species obtained from patients, and compare and contrast the strains present in Iraq with those already recognized worldwide.

2. Method

A cohort of 80 patients, ranging in age from 1 to 80 years, who presented to Al-Fayhaa Teaching Hospital, Al-Mawani Teaching Hospital, and Private Clinics in Basra province between March 2022 and September 2022, were the subjects of this aseptic collection of 40 blood and 40 stool samples, with or without symptoms of salmonellosis. Blood and stool samples were obtained and incubated in accordance with the standard protocol for sample collection in order to investigate microorganisms. For the proliferation of bacteria, specimens were collected in Brian Heart infusion broth for blood samples and selenite broth and/or tetrathionat broth for stool samples. These specimens were then transported to the laboratory of microbiology, where they were incubated at 37 ℃ for 24 hours [23]. The bouillon culture was streaked aseptically onto Xylose-Lysine-Deoxycholate (XLD) plates, which were subsequently examined for Salmonella-typical colonies after 18–24 hours of incubation at 37°C. Pure cultures were obtained by streaking suspect colonies onto nutrient agar plates. These cultures were subsequently subjected to biochemical testing using various media, including the Indole test, Kligler iron agar test, Citrate Utilization test, and Urea test. Every cultural medium is being prepared in accordance with the manufacturer's specifications.

2.1. Bacteriological diagnosis by VITEK 2

The Vitek2 system was used to identify the existing bacterial type (biomerieux, franch). The vitek2 system was used to diagnoses bacteria with a high degree of accuracy, it includes 47 tests of biochemical tests used in the diagnosis of bacteria.

2.2. Confirming Identification

All presumed isolates were validated through the implementation of molecular identification techniques utilizing 16S rDNA and sequencing. The genomic bacterial DNA was isolated in accordance with the PrestoTM mini g DNA Bacteria kit (Geneaid/ Taiwan).

2.3. Amplification of 16S rDNA gene by PCR technique

The complete 16S rDNA gene was amplified using the universal 16S rDNA primer set (F-5'-AGAGTTTGATCCTGGC-3' and R-5'-GGTTACCTTGTTACGACTT-3') in a conventional PCR reaction. Set as the volume of the PCR reaction was 50 µ. The sample comprised 19 µ of nuclease-free water, 2 µ (10 pmol) of forward and reverse primer, and 25 µ of 2X Taq Green Master Mix. It also included 2 µ of genomic DNA (DNA template). The reaction containers were subsequently placed in a thermocycler (Thermo, USA) for PCR. The following conditions were applied to the PCR: Following a PCR termination sequence consisting of the following: two minutes at 92^{o}C for initial denaturation, thirty cycles at 94^{o}C for denaturation for 30 seconds each, 45 seconds at 51.8^{o}C for annealing, 1.5 minutes at 72^{o}C for extension, and final extension at 72^{o}C for five minutes; the PCR product was subsequently run on 1% agarose accompanied by a DNA ladder. The DNA bands were observed using gel document, and digital photographs of the gel were taken [24] [24].

2.4. Sequencing of 16S rDNA

The 16S rDNA fragments were sequenced at Macrogen Company at site http://dna.macrogen.com (n=6). The 16S rDNA gene was processed using Mega11 software in order to obtain the nucleotide sequence. The BLAST N (Basic Local Alignment Search Tool, http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi) was utilized to analyze the PCR product sequence. BLAST N is an internet-based application hosted by the National Center for Biotechnology Information (NCBI) that determines the number of times subject sequences have been submitted to the International Nucleotide Database (e.g., DDJD, EMBL, GenBank, etc.) that matches the query sequence most closely. The identification of bacterial species names determined the similarity percentages, which ranged from 97% to 99% [25] [26].

2.5. Antimicrobial susceptibility testing.

The disc diffusion method was employed to assess the antimicrobial susceptibility of *Salmonella* spp. isolates in accordance with the protocols established by the Clinical and Laboratory Standards Institute (CLSI, 2022). A variety of antibiotics were employed against each isolate: Ciprofloxacin, Ampicillin, Cefotaxime, Azithromycin, Tetracycline, Trimethoprim-sulfamethoxazole, Trimethoprim-sulfamethoxazole, and Chloramphenicol. It is worth noting that all antibiotics utilized in this research were procured from Oxoid, a United Kingdom-based company.

2.6. Statistical Analysis

The data were analyzed using SSPS. The statistical significance of difference of data was assessed by chi square. P values $\leqslant 0.05$ were considered statistically significant.

3. Results

The findings revealed that among the eighty specimens of human excrement and blood that were examined, seven were positive for *Salmonella* serovars (7/80 or 8.75%).

3.1. Morphological characterization, cultural characters and biochemical test of Salmonella spp.

Characterization requires the isolation and identification of *Salmonella*; therefore, colonies exhibiting characteristic cultural features were selected as likely to be *Salmonella* spp [27]. Theoretical characteristics: Following a 24-hour incubation period at 37 ºC, the bacterium exhibited uniform, convex colonies measuring 2–4 mm in diameter. Figure 1 (A) illustrates how the pink

colonies of the *Salmonella* isolates produce H_2S, which darkens the colony's center to black. Figure 1 (B) demonstrates how the growth of the isolates in pallid selenite broth induces a reddening of the medium.

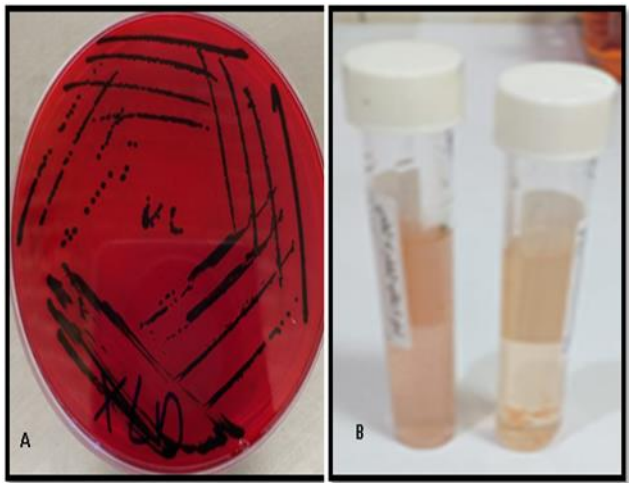


Fig. 1. Growth of Salmonella on XLD agar (A), Selenite broth (B), B-left: positive, B- right: negative.

Upon careful examination of the morphological and cultural attributes, it was ascertained that the organism in question is a *Salmonella* spp., as referenced in [28]. Consequently, seven clinical isolates exhibiting the aforementioned characteristics on XLD and selenite broth were classified as Salmonella spp. and subjected to additional validation tests, including biochemical analyses, 16S rDNA gene amplification, and DNA sequencing.

3.2. Biochemical test

Table 1, fig. 2 presents biochemical reactions of *Salmonella* spp.

Table 1. Biochemical reaction of *Salmonella* spp.

Biochemical test	Result
Oxidase	-
Indole	-
Ureas	-
Citrate utilization	+/-
Production of H2S	+

(+) Positive reaction, (–) Negative reaction.

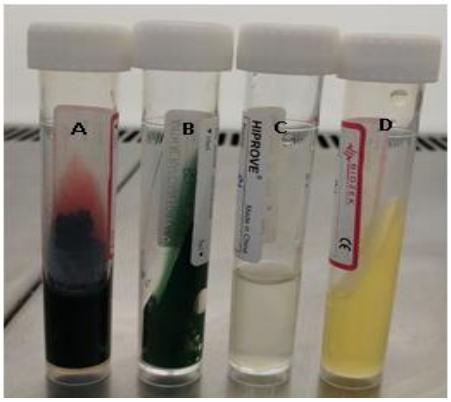


Fig. 2. Results of biochemical test isolate No.1 (*S.* Typhi): (A) Kilgler Iron test, (B) Citrate utilization test, (C) Indole test, and (D) Ureas test.

3.3. Confirmatory identification of Salmonella by use VITEK 2 system

The diagnosis of *Salmonella* was validated using the VITEK 2 system, which detects bacteria at the species level by utilizing 47 automated biochemical assays. As a concordance rate for confirmed diagnoses of bacteria at the species level, 95–99% has been established. The results obtained from the Vitek 2 system included six isolates that were identified as *S*. Typhi (see table 2) and one isolate that belonged to Salmonella spp.

Table 2. Analysis of Vitek 2 results for *S*. Typhi

NO. Of Samples	Vitek2 test														Isolates name
6	APPA	-	ADO	-	PyrA	-	IARL	-	dCEL	-	BGAL	-	O129R	+	*S.* Typhi
	H2S	+	BNAG	-	AGLTp	-	dGLU	+	GGT	-	OFF	+	GGAA	-	
	BGLU	-	dMAL	+	dMAN	+	dMNE	+	BXYL	-	BAIap	-	IMLTa	-	
	ProA	-	LIP	-	PLE	-	TyrA	+	URE	-	dSOR	+	ELLM	-	
	SAC	-	dTAG	-	dTRE	+	CIT	-	MNT	-	5KG	+	ILATa	-	
	ILATk	+	AGLU	-	SUCT	+	NAGA	-	AGAL	+	PHOS	+			
	GlyA	-	ODC	+	LDC	+	IHISa	-	CMT	+	BGUR	-			

3.4. Amplification of DNA by PCR technique.

As seen in fig. 3, the bacterial genome was recovered from each isolate using Kit Geneaid/Korea.

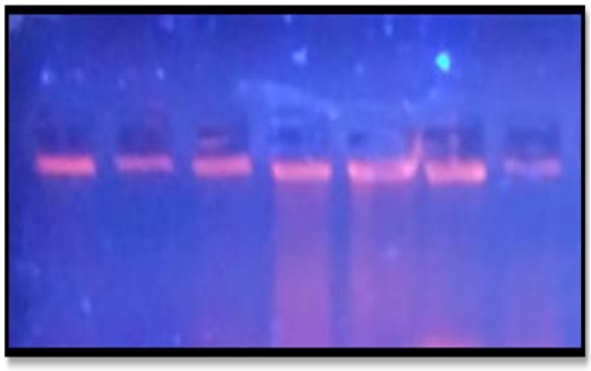


Fig. 3. Patterns of 0.8% of agarose gel electrophoresis shows genomic DNA bands of all *Salmonella* isolates visualized by UV light.

3.5. 16S ribosomal DNA

PCR was performed on the extracted DNA from all seven isolates that obtained from clinical specimens in order to amplify the 16S rDNA gene as in fig 3. As illustrated in fig. 4, the individual band of the amplified gene was characterized by an estimation of around 1500 base pairs (bp) using a standard molecular DNA marker of 2000 base pairs.

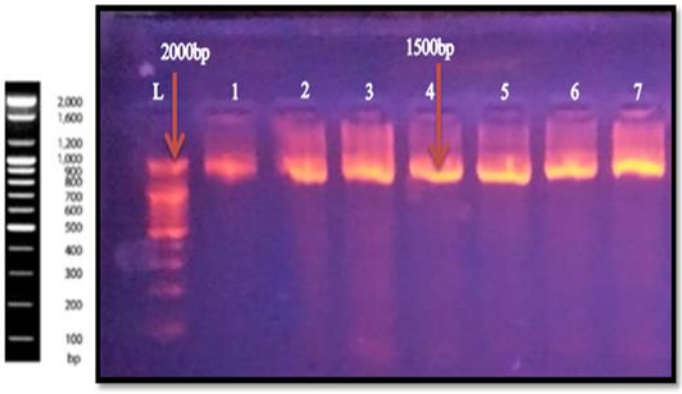
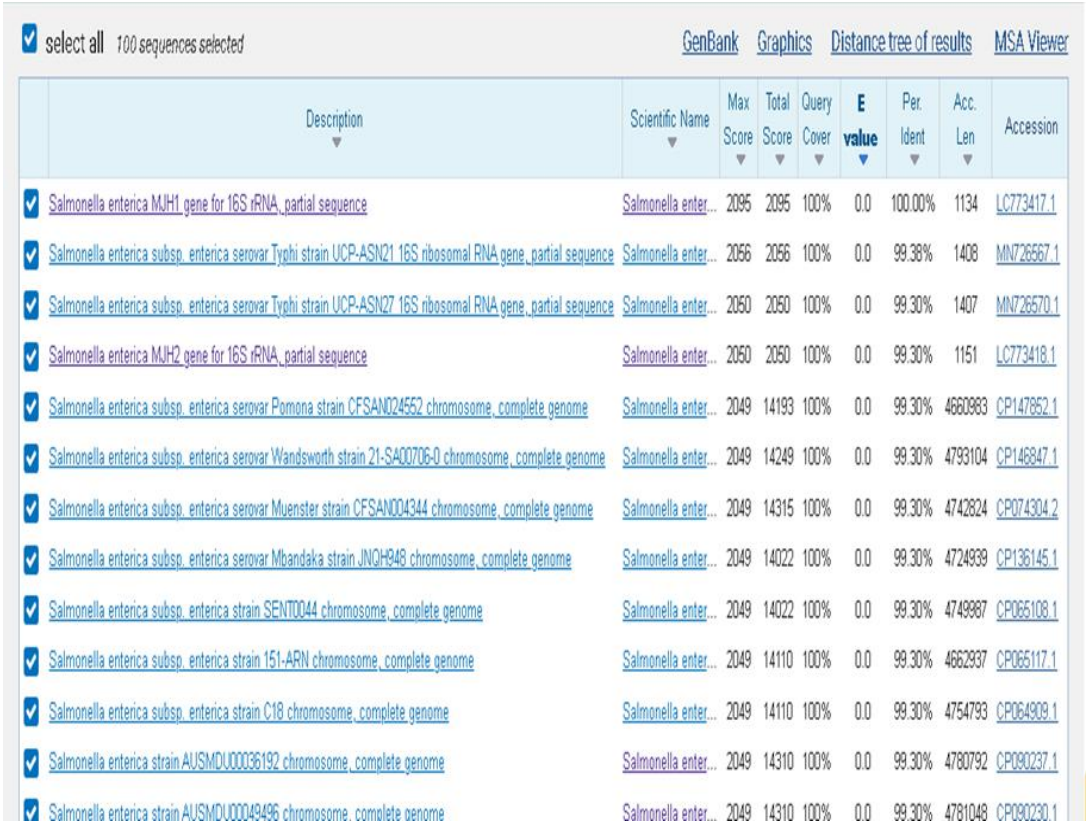


Fig. 4. Pattern of 2% agarose gel electrophoresis shows amplified 16S rDNA gene corresponding to ~ 1500 bp. Line L: 2000bp DNA ladder, lanes (1-7): 16S rDNA bands of bacterial isolates.

3.6. Sequence Analysis of 16SrDNA gene and Phylogenetic tree construction

In order to finalize the detection for *Salmonella* spp., the alignment information for the 16S rDNA gene was analyzed in order to determine the distribution of similarity with NCBI data. The confirmation of nucleotide set results was performed using the BLAST analysis, which is a Basic Local Alignment Search Tool (NCBI) tool. Alignment of sequences must be performed using GenBank-registered sequence databases containing information on the 16S rDNA genes of *S.* Enteritidis, *S.* Typhimurium, *S.* Typhi, and *S.* Paratyphi in order to determine the degree of similarity and identity of the 16srDNA gene and compare it to the sequences of the isolates under investigation. As illustrated in fig. 5, the outcomes demonstrate a high level of identity, query coverage, maximal score, and overall score. However, the e-value for other world *S.* Enteritidis, *S.* Typhimurium, *S.* Typhi, and *S.* Paratyphi is zero.



Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Salmonella enterica MJH1 gene for 16S rRNA, partial sequence	Salmonella enter...	2095	2095	100%	0.0	100.00%	1134	LC773417.1
Salmonella enterica subsp. enterica serovar Typhi strain UCP-ASN21 16S ribosomal RNA gene, partial sequence	Salmonella enter...	2056	2056	100%	0.0	99.38%	1408	MN726567.1
Salmonella enterica subsp. enterica serovar Typhi strain UCP-ASN27 16S ribosomal RNA gene, partial sequence	Salmonella enter...	2050	2050	100%	0.0	99.30%	1407	MN726570.1
Salmonella enterica MJH2 gene for 16S rRNA, partial sequence	Salmonella enter...	2050	2050	100%	0.0	99.30%	1151	LC773418.1
Salmonella enterica subsp. enterica serovar Pomona strain CFSAN024552 chromosome, complete genome	Salmonella enter...	2049	14193	100%	0.0	99.30%	4660983	CP147852.1
Salmonella enterica subsp. enterica serovar Wandsworth strain 21-SA00706-0 chromosome, complete genome	Salmonella enter...	2049	14249	100%	0.0	99.30%	4793104	CP146847.1
Salmonella enterica subsp. enterica serovar Muenster strain CFSAN004344 chromosome, complete genome	Salmonella enter...	2049	14315	100%	0.0	99.30%	4742824	CP074304.2
Salmonella enterica subsp. enterica serovar Mbandaka strain JNQH948 chromosome, complete genome	Salmonella enter...	2049	14022	100%	0.0	99.30%	4724939	CP136145.1
Salmonella enterica subsp. enterica strain SENT0044 chromosome, complete genome	Salmonella enter...	2049	14022	100%	0.0	99.30%	4749987	CP065108.1
Salmonella enterica subsp. enterica strain 151-ARN chromosome, complete genome	Salmonella enter...	2049	14110	100%	0.0	99.30%	4662937	CP065117.1
Salmonella enterica subsp. enterica strain C18 chromosome, complete genome	Salmonella enter...	2049	14110	100%	0.0	99.30%	4754793	CP064909.1
Salmonella enterica strain AUSMDU00036192 chromosome, complete genome	Salmonella enter...	2049	14310	100%	0.0	99.30%	4780792	CP090237.1
Salmonella enterica strain AUSMDU00049496 chromosome, complete genome	Salmonella enter...	2049	14310	100%	0.0	99.30%	4781048	CP090230.1

Fig. 5. Gb-NCBI blasting of local sequence 16S rDNA gene with world sequences of 16S rDNA gene.

3.7. Recording Iraqi Salmonella serovars isolates in NCBI based on 16SrDNA gene

After DNA sequencing partial nucleotide of 16S rDNA from each bacterial isolate was obtained, six sequence of Salmonella spp were isolated from human in Basrah Province. All these sequences accepted in gene bank and each sequence takes accession number (LC773417.1), (LC773418.1), (LC773419.1), (LC773420.1), (LC773421.1) and (LC773422.1). The results of this study were recorded and published in the International Nucleotide Sequence Database Collaboration (INSDC), this location covers the database of National Center for Biotechnology Information (NCBI) as in fig. 6. A total of six accession numbers were derived from 16S rDNA sequences, and these were registered at the gene bank-NCBI. Among these, (LC773417.1), (LC773418.1), (LC773419.1), and (LC773420.1) correspond to S. Typhi, while one (LC773421.1) is associated with S. Typhimurium. However, one accession number (LC773422.1) was exclusively identified as Salmonella enterica.

Fig. 6. Accession number report of local sequence LC773417.1

3.8. Phylogenetic analysis

A phylogenetic tree was constructed using the maximum composite likelihood approach to analyze the nucleotide sequences of four examined strains and eight reference strains. The resulting tree displayed the evolutionary distances between these strains. Figure 7 displays the evolutionary relationships between the examined strain and the reference strains, along with the determined genetic distance among them. The examination of the phylogenetic tree revealed the presence of 5 primary clades. All examined strains exhibited distinct variations in their sequencing. The present results revealed that the studied strain LC773418 showed high similarity with two sequences, OU943338 in United Kingdom and CP090237 in Australia with genetic distance or sequence divergence 1.85430 reflecting a close relationship of these strains. Also, another studied strain LC773417 showed similarity with MT509427 in Bangladesh, CP136145 in China, CP074304 in USA and CP065108 in Taiwan strains with genetic distance 1.56510 reflecting a close relationship of these strains. Furthermore, studied strain LC773420 showed similarity and cluster with OQ085110 in Islamabad-Pakistan strain with genetic distance 1.73878. Interestingly, the last studied strain LC773419 was isolated alone in one clade, with genetic distance 2.37497 from another studied strain LC773420. Also, reference strain MN726567 in Lahore-Pakistan was isolated alone in one clade with genetic distance 4.02628 reflecting a clear variation in their sequences.

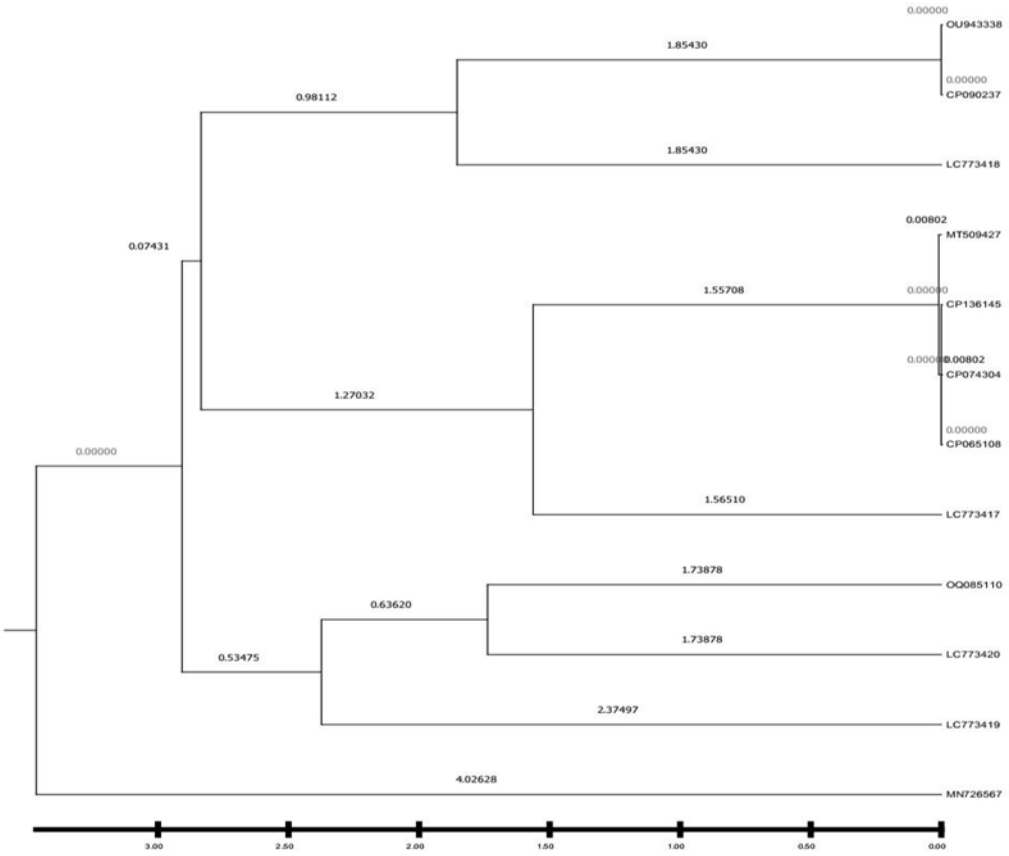


Fig. 7. Molecular Phylogenetic Tree of *S.* Typhi based on 16S rDNA gene, illustration by using Maximum Likelihood method.

3.9. Antibiotics susceptibility test

A total of seven *Salmonella* serovars isolates were evaluated against the subsequent antibiotics in this investigation: Chloramphenicol, Ampicillin, Cefotaxime, Azithromycin, Ciprofloxacin, Tetracycline, Trimethoprim-sulfamethoxazole, and Cefotaxime. *Salmonella* spp. contained 42.86% (three out of seven isolates) MDR bacteria and 57.14% (four out of seven isolates) extensively drug resistant bacteria (see figure; there were no statistically significant differences (P=0.162). The antibiotic usage frequency of *Salmonella* isolates is presented in tab. 3.

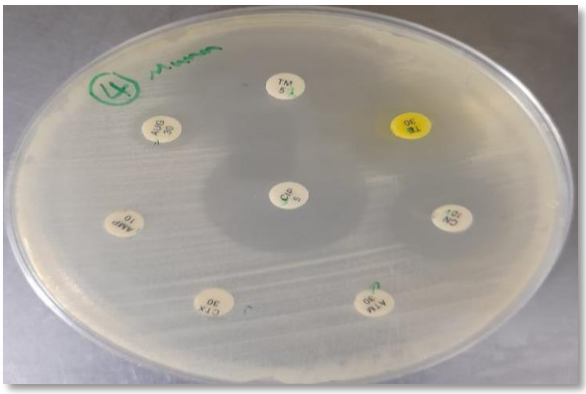


Fig. 8. Result of disc diffusion method for *S.* Typhi (isolate No. 4)

Table 3. Frequency of antibiotics susceptibility of *Salmonella* serovars

Antibiotic disc	No. (%) of sensitive	No. (%) of intermediate	No. (%) of resistant	P value
Ampicillin	0	0	7(100%)	------
Cefotaxime	0	0	7(100%)	------
Azithromycin	0	4(57.14%)	3(42.86%)	0.705 NS
Tetracycline	2(28.57%)	1(14.29%)	4(57.14%)	0.520 NS
Ciprofloxacin	0	3(42.86%)	4(57.14%)	0.705 NS
Trimethoprim-sulfamethoxazole	1(14.29%)	3(42.86%)	3(42.86%)	0.712 NS
Chloramphenicol	1(14.29%)	3(42.86%)	3(42.86%)	0.712 NS*

NS*= no significant differences

4. Discussion

Eighty human samples (blood and stool) were obtained for this investigation; 8.75% of the samples tested positive for Salmonella. Numerous national and local studies conducted in Iraq have examined the diagnosis and spread of bacteria in a variety of methods; for instance, in one study from the city of AL-Samawah, 23.7% of positive samples (45 out of 190) contained Salmonella [29]. Another investigation conducted in Babylon Province revealed that 18.66% (14 out of 75) of the 75 samples contained Salmonella [30]. According to a study conducted in Nigeria, the proportion of Salmonella-positive isolates was as follows: out of 155,837 clinical samples, 1,967 (41.9%) originated from stool and 2,732 (58.1%) were blood samples [31]. The similarities and differences between this study and others are attributable to a variety of diagnostic techniques, health consciousness, and sanitation adherence.

Due to the rapid increase in Salmonella infections, it is necessary to develop dependable and rapid techniques for the immediate detection of the pathogen in order to initiate the necessary control measures. An inherent constraint of phenotypic methods for bacterial identification is the occasional inability to determine the species-level identification of the bacterium [30].

In this study, diagnosis using biochemical tests (Vitek system) led to the diagnosis of most isolates as S. Typhi, and one isolate was diagnosed at the genus level only, as the genus Salmonella contains many serovars that are close to each other in their biochemical fingerprint. Several studies have found that accurate bacterial identification is primarily dependent on the metabolic fingerprint of the isolates, which fluctuates according to the physiological state of the isolate during examination [32]. In recent times, significant advancements in research and development, coupled with the extensive implementation of PCR (Polymerase chain reaction), cloning, and DNA sequencing, have rendered 16S rDNA gene sequencing indispensable for the precise identification of bacterial isolates and the identification of novel species.

A multitude of investigations have demonstrated that the 16S rDNA gene sequences of the majority of presently documented species diverge from closely related species within the same genus by a minimum of 1% of sequence positions, and frequently by a greater margin [33]. Drancourt et al. established cutoff value for the identification of microbes using 16S rDNA. In the context of identifying bacterial species, a suitable cutoff is a similarity value of at least 99% for 16S rDNA gene sequences. For genus-level identification, a similarity value of at least 97% is acceptable [34]. In this regard, the distribution of domestic strains among Iraqi strains was satisfactory for the United States, China, the United Kingdom, Australia, Bangladesh, and Pakistan. The findings of this study indicate that certain strains of *S.* Typhi from Iraq exhibit a significant genetic distance from the national strain previously documented in NCBI. This genetic divergence can be attributed to mutations that have occurred within these strains.

Antibiotic sensitivity testing was conducted, yielding the following results: 42.86 percent MDR bacteria and 57.14 percent XDR bacteria. This was consistent with the findings of other investigations [35] [36] that all *Salmonella* isolates exhibited resistance to at least one antibiotic, as determined by their antibiograms.

According to the study [37], the proportion of multidrug-resistant bacteria was 42.68%, or 35 out of 82. A significant proportion of MDR *Salmonella* was identified, as reported by Salman et al [38]. One possible explanation for the elevated incidence of MDR is the widespread prescription of antibiotics without medical consultation, a practice that is prevalent in developing nations [39], specifically Iraq [38].

5. Conclusion

Although VITEK2 diagnosis is accurate and performed at the species level, it is not as accurate as genetic amplification diagnosis since VITEK 2 diagnosis does not always diagnose at the species and serovars level for *Salmonella*. PCR demonstrated a notable degree of precision when it came to identifying *Salmonella* at serovar level. The identification of bacteria with both multidrug resistance and extensive drug resistance serves as a warning sign for the need to decrease antibiotic usage. Additionally, phylogenetic analysis showed that some isolates of *S.* Typhi had clear variation from some comparative reference isolates registered in NCBI.

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الشجرة الوراثية لجرثومة *Salmonella enterica* ذات المقاومة للعديد من الادوية والواسعة المقاومة المعزولة من مرضى داء السالمونيلا في محافظة البصرة العراق.

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معلومات البحث

الاستلام 30 نيسان 2024
المراجعة 2 تموز 2024
القبول 13 تموز 2024
النشر 31 كانون الأول 2024

الكلمات المفتاحية

مقاومة للأدوية على نطاق واسع، داء السالمونيلات، شجرة النشوء والتطور، مقاومة الأدوية المتعددة.

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الملخص

السالمونيلا،هو جنس جرثومي، مسؤول عن داء السالمونيلا، وهو مرض عالمي يمكن أن يصيب ع البشر والحيوانات. تم جمع 80 عينة سريرية من أفراد تظهر عليهم أعراض مرض السالمونيلا في محافظة البصرة. تم الحصول على عزلات السالمونيلا وتحديدها باستخدام الزراعة الجرثومية في ظروف مناسبة وتقنيات معتمدة، مثل نظام Vitek2 وتضخيم الجينات 16S rDNA. أجريت دراسة لتقييم مدى حساسية المضادات الحيوية باستخدام إرشادات CLSI 2022. النتيجة: أشارت النتائج إلى أن 7 عينات من أصل 80 عينة سريرية تم فحصها كانت إيجابية للسالمونيلا. اعتمادًا على الطريقة المستخدمة لتسلسل نيوكليوتيدات جين 16S rDNA، تم تقديم ستة عزلات فقط لتسجيل تسلسلاتها في Gene Bank-NCBI من أجل تشخيصها على مستوى الأنواع وتحت النوع، والحصول على أرقام الانضمام، ومن ثم تحديد السلالات الخاصة بها. يتم تمثيل السالمونيلا التيفية بأربعة أرقام انضمام (LC773417.1)، (LC773418.1)، (LC773419.1)، و (LC773420.1). تتم الإشارة إلى السالمونيلا تيفيموريوم برقم انضمام واحد LC773421.1. تم إدراج رقم مُدخل واحد LC773422.1 تحت اسم السالمونيلا المعوية فقط. الخلاصة: أظهر تفاعل البوليميراز المتسلسل درجة ملحوظة من الدقة عندما يتعلق الأمر بتحديد السالمونيلا على مستوى تحت النوع. تم بنجاح عزل وتحديد سلالات السالمونيلا التي تظهر مقاومة متعددة وواسعة النطاق للأدوية.

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