Genetic and Biochemical Detection of *Salmonella enterica* Isolated from Patients Suffering Watery Diarrhea and Typhoid Fever in Babylon Province

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Abstract

Background: Salmonellosis is a severe infectious zoonotic disease, which increases the importance of identifying and controlling the causative strains. **Objectives:** This study aimed to develop a rapid molecular diagnostic test to determine and purify bacterial isolates based on the specific primer. SE1472298-2 for *Salmonella* serovar *enteritidis*; gene STM4497 demonstrated specificity for *Salmonella* serovar *typhimurium* and gene O antigen synthesis *tyv* for *Salmonella* serovar *typhi* isolated from patients in Babylon province, Iraq. **Materials and Methods:** Two hundred clinical stool specimens were collected from patients suffering from watery diarrhea. Blood specimens obtained from patients with typhoid fever who were admitted to three hospitals of Babylon Governorate. **Results:** There were a total of 200 samples; 34 (17%) were discovered by biochemical tests. The diagnosis of these samples was confirmed by polymerase chain reaction, depending on the target gene, so the number of isolates was 25 (73.53%), *Salmonella enteritidis* 13 (52%) *Salmonella typhimurium* 6 (24%), and *Salmonella typhi* 6 (24%). **Conclusion:** Molecular techniques, particularly polymerase chain reaction, can rapidly and precisely identify Salmonella isolates.

Keywords: *S. enterica*, *SE1472298-2*, *STM4497*, *tyv* gene target, PCR

Introduction

Salmonella enterica is a gram-negative, motile, facultatively anaerobic, rod-shaped bacteria belonging to the Enterobacteriaceae family.[1]

Infections caused by *S. enterica* continue to be one of the most prevalent foodborne illnesses, which is a concern for public health on a global scale.[2] *Salmonella* was identified as a significant zoonotic disease with economic ramifications as early as the 1950s by the United Nations Food and Agriculture Agency and World Health Organization. These organizations are part of the United Nations.[3] *Salmonella* bacteria belong to the genus *Salmonella* and are classified into two species: *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* may also be classified into six different subspecies, and it has more than 2600 different serovars.[4] Salmonella's pathogenesis is mediated by a number of genes that enhance the invasion of host cells, intracellular survival, and colonization of the host.[5]

Salmonella is the most common cause of foodborne disease and is responsible for thousands of fatalities worldwide. It is the most significant member of a huge genus important to public health globally.[6] *Salmonella* is one of the organisms that cause severe acute diarrhea in children under 5 years of age.[7] *Salmonella* are rod-shaped, gram-negative bacteria that are facultative anaerobes and belong to the family Enterobacteriaceae. *Salmonella* can only live in the absence of oxygen. There are around 2600 serovars that belong to *S. enterica*, and they have been described all over the world. Several of these serovars are capable of causing

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infections in people as well as animals.[8] *Salmonella* strains are nonspore producing, primarily motile enterobacteria with cell diameters ranging from approximately 0.7 μ to 1.5 μ, lengths ranging from 2 μ to 5 μ, and peritrichous flagella. *Salmonella* species are responsible for a wide range of human and animal diseases.[9]

Salmonella infection or a condition linked with it, salmonellosis is most frequently characterized by enteritis; gastroenteritis is typically associated with non-typhoidal *Salmonella* serovars such as typhimurium (*S. typhimurium*) and enteritidis (*S. enteritis*) and is the most prevalent symptom of salmonellosis.[10]

Typhoid fever is a life-threatening systemic illness that is largely caused by the *S. enterica* serovar Typhi (*S. typhi*), and it continues to be a substantial public health concern in resource-poor settings, such as in some regions of Asia and Africa.[11] Confirmation of typhoid was through identifying *S. typhi* organisms in blood and stool cultures in the second week of infection.[12]

Materials and Methods

Samples collection

Two hundred clinical stool and blood specimens were collected from patients in the stages of all ages and both sexes suffering from watery diarrhea or with (mucus, pus, little blood or no) and blood specimens obtained from patients with typhoid fever were admitted in three hospitals of Babylon Governorate: the Imam Al-Sadiq Teaching Hospital, Babylon Surgical Hospital, and Babylon Teaching Hospital for Maternity and Children throughout 10 months (from February 2022 to November 2022).

Culture of specimens

Distribute 10 μL (loop entire) of previously inoculated and incubated brain heart infusion broth and tetrathionate broth on xylose lysine deoxycholate agar (XLD) plates and incubate at 37ºC overnight (18–24 h). The plates were examined based on colonial phenotyping. Salmonella suspected colonies on XLD aseptically transferred to Triple Sugar Iron agar slants, urea agar slant, citrate agar, and MacConkey agar for further confirmatory identification by VITEK 2 compact system, and molecular diagnosis using polymerase chain reaction (PCR) technique.

Extraction of DNA from bacteria

The extraction of DNA was performed using purification kit, which was supplemented by a product of the manufacturing firm (Geneaid, UK).

Polymerase chain reaction amplification

Molecular identification of *S. enterica* strains was made by using gene *SE1472298-2* for *S. enterica* ser. *Enteritidis*, gene *STM4497* demonstrated specificity for *Salmonella enterica* ser. *Typhimurium* and gene O antigen synthesis *tyv* for *Salmonella enterica* ser. *Typhi* as shown in [Table 1].

Molecular detection of *Salmonella enterica* using Uniplexpolymerase chain reaction

For the amplification of *S. enteritidis*, *S. typhimurium*, and *S. typhi*, a Uniplex-PCR was done with a total volume of 25 μL (Primers 1.5×2, DNA 3 μL, Master mix 12.5 μL, RNAse free water 6.5 μL). The primers were used for this reaction using GoTaq® G2 Green Master Mix (Promega, USA). A 5 μL of the PCR products amplicon was loaded into 2% agarose gels in 1× TAE and ran at 100 v in 1× TAE for 40 min. The gel documentation system photographed the gels. The PCR products for *S. enteritidis*, *S. typhimurium*, and *S. typhi* were 612 bp, 523 bp, and 614 bp, respectively.

Ethical approval

Before samples collection, each participant was provided with information, and their verbal consent was obtained. Under the reference number BMS/0231/016, the committee at the College of Medicine, University of Babylon, Iraq, gave their stamp of approval to the publishing ethical research project.

Table 1: Primer sequences of all studied genes in this study with their amplicon size (bp) and reference

Primer name	Primer sequence (5'-3')	Size bp	PCR condition	Reference
STM4497 F	GGAATCAATGCCCGCCAATG	523	94ºC 5 min 94ºC 60 s 62ºC 2 min 35× 72ºC 2 min 72ºC 10 min	[13]
STM4497 R	CGTGCTTGAATACCGCCTGTC			
SE1472298-2 F	CTTGGAGAGCTGCGCTAAAG	612	94ºC 5 min 94ºC 60 s 62ºC 2 min 35× 72ºC 2 min 72ºC 10 min	[14]
SE1472298-2 R	TAAGGCACCTCTCAACACTG			
Tyv F	GAGGAAGGGAAATGAAGCTTTT	614	95ºC 5 min 95ºC 30 s 55ºC 45 s 30× 72ºC 2 min 72ºC 8 min	[15]
Tyv R	TAGCAAACTGTCTCCCACCATAC			

Results

All of the samples were put through aerobic culturing on selective media. Out of the total, there were 200 specimens; 180 showed positive bacterial culture, while the remaining 20 did not show any growth. This suggests the presence of microorganisms that are difficult to culture, such as viruses, fungi, and other agents, or it may be due to differences in the size and nature of the specimens.

Only 34 (17%) of the positive culture specimens showed positive results on culture and were identified as *S. enterica*, as shown in Table 2. The results showed that only 25 (73.5% out of 34) were positive for culture and biochemical tests, and among these 25 isolates, 13 (52%) were *S. enteritidis*, 6 (24%) were *S. typhimurium*, and 6 (24%) were *S. typhi*. These 25 isolates are depicted in Figures 1–3.

Discussion

The previous study obtained by Aljobouri[16] discovered that 17.5% were diagnosed as *S. enterica* isolates based on the results of the cultures of specimens. Also, results obtained by Andoh *et al*.[17] found that (16.05%) were identified as *S. enterica*.

The study conducted by[18] in Thi-Qar hospitals mentioned that Salmonella accounts for (11.17%) of diarrheal cases. Another study[19] showed that the prevalence of Salmonella enterica was 8%.

The variation in the isolation rate of *S. enterica* between studies depends on several factors such as virulence of isolates, the health status of patients and the effect of environmental conditions, isolation and identification procedures, and the socioeconomic and cultural level of patients. It may be due to differences in the size of the samples.[16]

Salmonellosis remained one of the three most common meat-associated diseases in humans.[20] The chance of infections with *Salmonella* spp. increases owing to the fact that animal products are easily contaminated with bacteria and stimulate their development if they are not properly handled, processed, and conserved[21] Salmonellosis is the most common and important zoonotic disease.[22]

On the other hand, using antimicrobials causes a temporary rise in the chance of infection upon exposure to a foodborne pathogen, such as *Salmonella* spp., as well as a loss in an individual's resistance to colonization with these non-commensal bacteria.[23]

Salmonella enteritidis and *S. typhimurium* are the organisms most commonly found to be responsible for the majority of Salmonella infections linked to the eating of infected chicken and beef products.[24] In areas lacking clean drinking water and sanitation facilities, typhoid disease significantly threatens public health.[25] Salmonella contamination of chicken products is possible at numerous stages of the food manufacturing process, including production, processing, distribution, retail marketing, handling, and preparation.[26]

The VITEK 2 system is a confirmatory phenotypic tool that can give correct microbiological identification. It is the most automated platform available, with speedy results, high specificity, proven confidence, and less training time than manual microbial identification procedures.

To confirm the diagnosis of *S. enterica* ser. *typhimurium* based on STM4497, *S. enterica* ser. *enteritidis* based on

Table 2: The frequency of *Salmonella enterica* compared to other etiological agents associated with the isolated sample

No. sample	Culture and VITEK 2 compact			Molecular diagnosis	
	S. enterica	Negative results	Other bacteria and fungi	Positive results	Negative results
200	34 (17)%	146 (73)%	20 (10)%	25 (73.53)%	9 (26.47)%

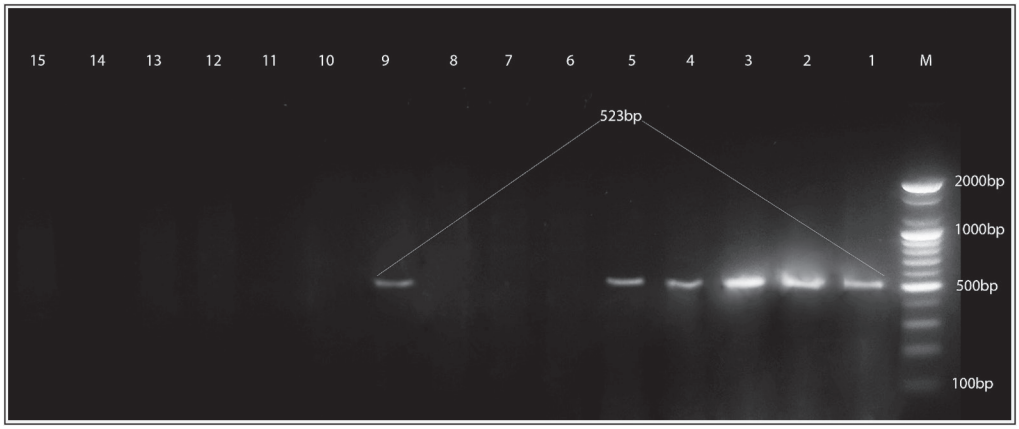

Figure 1: Agarose gel electrophoresis of PCR product obtained with *Salmonella typhimurium*—a specific primer that generated 523 bp amplicon; lanes: 1–5 and 9 are positive, others are negative. M lane is DNA ladder 100 bp

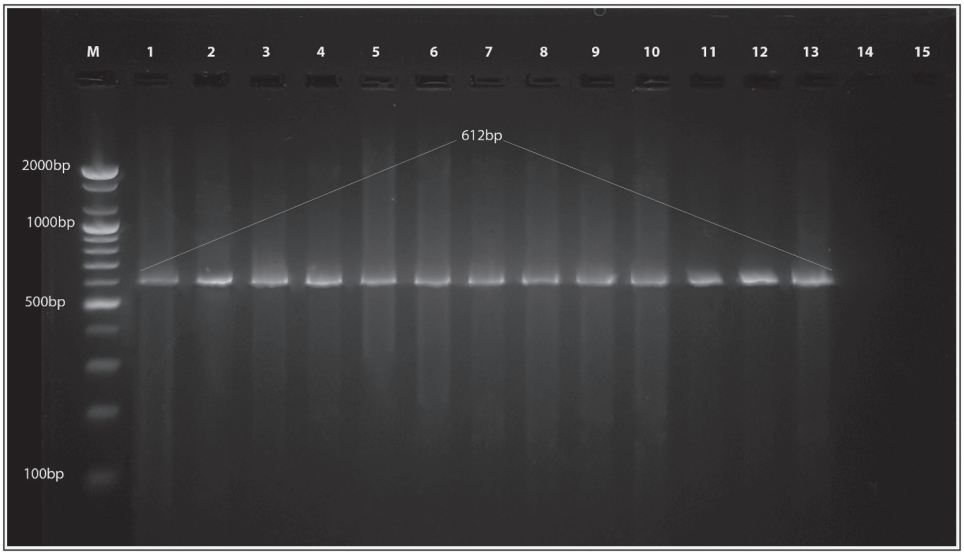

Figure 2: Agarose gel electrophoresis of PCR product obtained with *Salmonella enteritidis*—a specific primer that generated 612 bp amplicon, Lanes: 1–13 are positive, others are negative. M lane is DNA ladder 100 bp

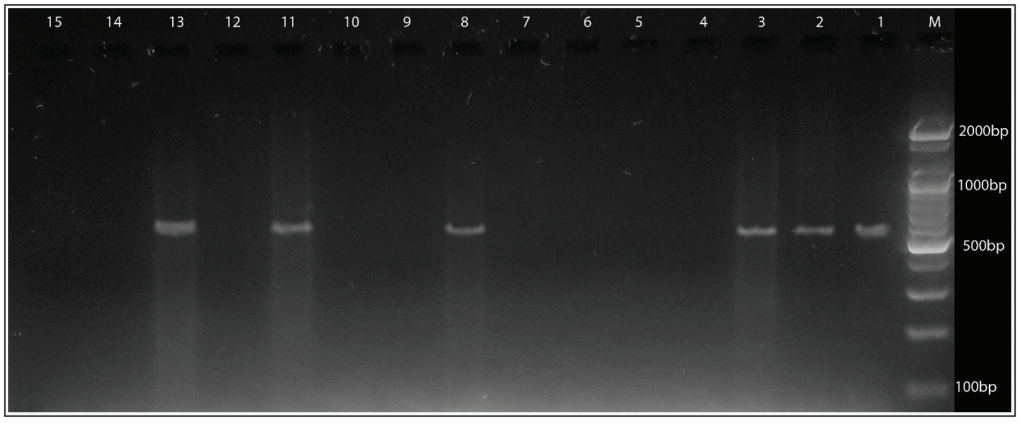

Figure 3: Agarose gel electrophoresis of PCR product obtained with *Salmonella typhi*—a specific primer that generated 614 bp amplicon, Lanes: 1–3, 8, 11, and 13 are positive, and others are negative. M lane is DNA ladder 100 bp

SE1472298-2, and *S. enterica* ser. *typhi* based on O antigen synthesis *tyv* in Uniplex-PCR.

Salmonella enteritidis was most frequently identified in human cases; the present study was correlated with a study obtained by Rodulfo *et al.*,[27] who reported that *S. enteritidis* was the most common.

The findings of this research, which were achieved via the use of molecular techniques, are as follows: 13 (52%) from *S. enteritidis*, 6 (24%) from *S. typhimurium*, and 6 (24%) from *S. typhi* out of 34 specimens were positive results for *S. enterica*. Previous study results obtained by[28] who it was discovered had *S. enteritidis* (23%) and (32%) were identified as carriers of *S. typhimurium*. While the study by Aljobouri[16] who were found that 17 (70.83%) isolates of *S. typhimurium* and 7 (29.16%) isolated *S. enteritidis*.

Another study[29] showed that 10 (33.3%) from *S. typhimurium* and 8 (26.6%) from *S. typhi*.

To identify Salmonella more quickly, certain molecular technologies, particularly ones that are both speedy and have a high level of repeatability, have been developed. Because of their increased ease of use, speed, repeatability, accuracy, and cost, PCR and other nucleotide-based technologies have emerged as potentially strong alternative approaches in microbiological diagnostics.[30]

Nevertheless, this method will improve the accuracy, sensitivity, specificity, and cost-effectiveness of the detection of *S. enterica* compared to the culture technique, making PCR the best option for diagnosing *S. enterica* infection. Nevertheless, the molecular approach has an advantage over convection techniques in that it may produce findings within 24 h, whereas traditional culture followed by biochemical testing requires 24–48 h.

The diagnosis of typhoid fever is determined using standard culture techniques and biochemical assays. Unfortunately,

the traditional procedure for diagnosing typhoid fever needs at least 4 or 5 days to get promising findings. The diagnosis of typhoid fever requires a quick, alternative procedure.[31] Molecular biology-based approaches, such as PCR tests, have been described for the quick, specific, and sensitive identification of microorganisms in a variety of clinical samples. One example of such a technique is the polymerase.[32,33]

Conclusion

PCR is one of the molecular methods that may be used to quickly and accurately identify Salmonella isolates better than traditional culturing methods, because it gives accurate results and rapid diagnosis compared to traditional methods.

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Conflicts of interest

There are no conflicts of interest.

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