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Isolation and Molecular Identification of Non-Typhoidal Salmonella from Humans with Diarrhea

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Abstract:

This investigation was done for isolation and molecular identification of non-typhoidal salmonella from humans. From 2021 till end of 2022, 100 stool samples were collected from humans with diarrhea different clinics of Baghdad province. After 24 hours of incubation at 37 °C, the samples were grown on several selective medium to identify salmonella colonies. Multiple biochemical analyses were performed on the growth isolates, and the results were verified using the Api20-E system. Colonies having biochemical properties consistent with Salmonella spp. were examined using the API20-E method, and their identities were confirmed using PCR targeting 16S rRNA. Six (6%) human *Salmonella enterica*serovars isolates were positively subspecies-identified by routine bacteriological method. In this work, the 16S rRNA gene was used to screen for Salmonella spp. in isolates from diarrhoea samples. A total of 6 *S. enterica* isolates were taken from people, and 6 (6%) of those isolates showed positive amplifications in the first round of PCR, which included running the 16S rRNA 1500bp gene.

In conclusion, non typhoidal salmonella were isolated and identified in a significant percent in human with diarrhea.

Keywords: salmonella, non typhoidal, diarrhea, human, molecular.

Introduction:

Many different types of Salmonella may cause food poisoning in both humans and animals. Non-typhoidal Salmonella is responsible for about 93.8 million cases of gastroenteritis each year. This is an international health concern [1]. Humans face a significant danger from Salmonella enterica. Multiple virulence factors are used by these microorganisms to infect humans [2]. Most cases of food poisoning in a number of countries are caused by Salmonella entericaTyphimurium (ST). Although most infections with this bacterium result in self-limiting gastroenteritis, it may sometimes cause more serious diseases [3]. It was classified in 2012 as a zoonotic, not species-specific, bacterium due to its capacity to spread illness across animals and humans.This bacteria should be able to survive in the stomach's acidic environment. To survive, it activates a mechanism called the acid tolerance response (ATR), which keeps the pH within the cell from falling below the pH outside [4].

In order to infect the epithelium, Salmonella spp. must first break through the mucus layer already present in the intestinal wall [5, 6]. Salmonella species cause a clinical state characterized by diarrhea, electrolyte loss, and inflammation of the digestive system by interacting with the epithelium [6]. There are many genes on the bacterial chromosome that code for virulence factors and provide bacteria of the same family same features; these genes are termed housekeeping genes.

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Similar genes to those discovered on pathogenicity islands are also present in mobile genetic elements such transposons, plasmids, and bacteriophages [7]. S. enterica has a number of virulence genes, the products of which contribute to the pathogen's pathogenicity in the host [8]. These genes include *Inv*, *sef*, and *pef*, all of which are involved in pathogen attachment and invasion.

One of the most common types of zoonotic infections in kids is non-typhoidal Salmonella (NTS), which may be caught either eating or drinking tainted food or water or by having direct contact with animals or poultry and causing diarrhea[9]. The Enterobacteriaceae (Salmonella) *S. Typhimurium* and *S. enteritidis* are two of the most common serotypes associated with NTS gastroenteritis in children over the globe[10].

Certain bacteria feature fimbriae on their cell surfaces, and these structures have been linked to colonization and the onset of host infection [11]. Salmonella enterotoxin gene (stn) is present in many Salmonella serotypes and Salmonella strains [12]. The *stn* gene was selected because of its excellent specificity and conservation across Salmonella entericaserovars. Salmonella contributes to the development of diarrhoea and fever through the presence of a number of virulence factors, including the ability to adhere to intestinal epithelial cells via cilia, flagella, and biofilmae, resistance to antibiotics, and the ability to avoid being ingested by the body's immune system (phagocytosis), and the presence of heat-labile toxins [13].

This investigation was done for isolation and molecular identification of non-typhoidal salmonella from humans.

Materials and Methods:

From 2021 till end of 2022, 100 stool samples were collected from humans with diarrhea different clinics of Baghdad province. After 24 hours of incubation at 37 °C, the samples were grown on several selective medium to identify salmonella colonies. Multiple biochemical analyses were performed on the growth isolates, and the results were verified using the Api20-E system. Colonies having biochemical properties consistent with Salmonella spp. were examined using the API20-E method, and their identities were confirmed using PCR targeting 16S rRNA, which selected according to[14].

The primer designed **F** AGAGTTTGATCCTGGCTCAG, **R** GGTTACCTTGTTACGACTT, the base per was 1500.

Six different strains of Salmonella were used to get genomic DNA. After that was done, 10 ml of nutritious broth medium was put into the bacterial culture and it was left in a 37 °C, shaking incubator for the night. After preparing agarose gel electrophoresis per [15], the final product was observed by adding the remaining components to the reaction mixture. After taking pictures of the agarose under the UV transilluminator, the agarose was taken out of the tank.

Results and discussion:

Six (6%) human *Salmonella enterica*serovars isolates were positively subspecies-identified by routine bacteriological method. In this work, the 16S rRNA gene was used to screen for Salmonella spp. in isolates from diarrhoea samples. A total of 6*S. enterica* isolates were taken from people, and 6 (6%) of those isolates showed positive amplifications in the first round of PCR, which included

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running the 16S rRNA 1500bp gene (Figure 1).

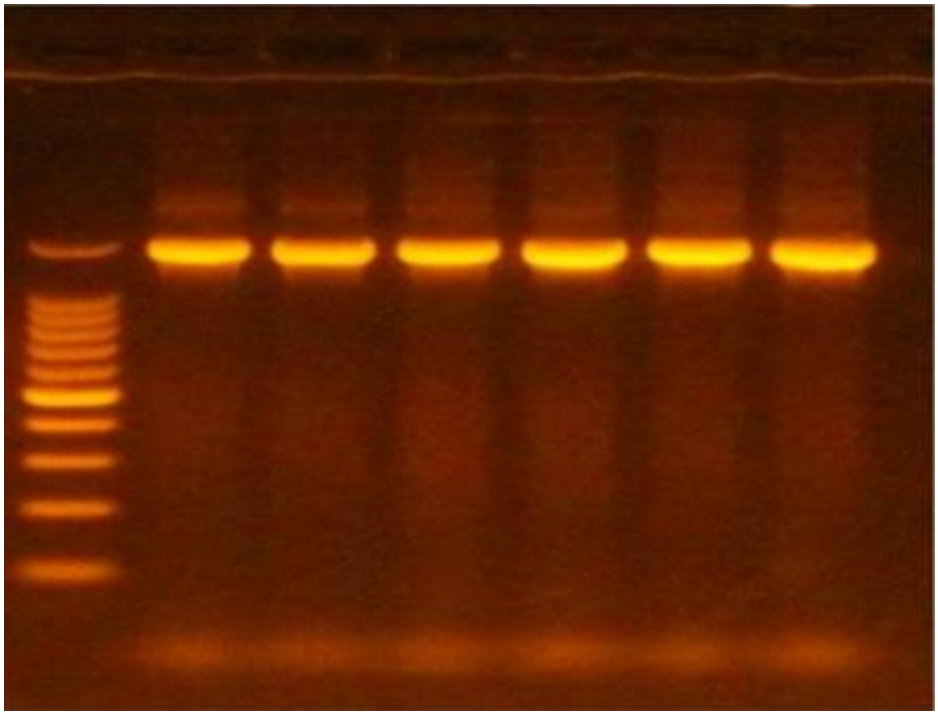

Figure 1. The 1500 base pair (16s rRNA gene) PCR product was run in 1% agarose at 100V for 1 hour.

Salmonella is blamed for a disproportionate share of the deaths and illnesses caused by contaminated food in third world nations [1]. Many investigations have been conducted in various parts of Iraq; however, the percentages of Salmonella isolation remain approximative [16, 17]. Among 400 kid stool samples obtained in Thi-Qar [18], only 20 individuals were positive (5%), which is consistent with the present research, and with study [19], that reports that sixteen percent (1.6) of Salmonella tests were positive out of a total of one hundred samples. Even more so, our results in Basra are consistent with those of [20].

Because this bacteria has been researched at different periods and in different locales, it's likely that the huge variation in isolation rates may be explained. As a result of variations in climate, geography, and hygiene, as well as environmental contamination and the improper use of medications, the disease's signs have varied throughout time and space. The unhygienic environment also has a role.

The majority of bacteria are identified by their phenotypic characteristics. However, the PCR approach is necessary for the precise identification of Salmonellaspp. in certain situations, especially when unusual culture features arise (21). Both the physical similarities among these creatures and the necessity for an expert make these procedures unreliable for species determination. Controversy surrounds the interpretation of results from these techniques because of their low sensitivity and specificity. The PCR technique of in vitro amplification of DNA is a potent tool in microbiological diagnostics (22).

We use the 16S rRNA method, the current gold standard for determining the genus level of bacteria (23). The findings demonstrated that 1500 bp (16S) rRNA was present in 6 of isolates. Several

publications, including (24 and 25), observed that all Salmonella species isolates were positive to the 16s rRNA gene, therefore the findings of our research are closely comparable to theirs. One potential method for the speedy identification of Salmonella bacteria is the targeting of genes for detection (24).

Conclusion:

Non typhoidal salmonella were isolated and identified in a significant percent in human with diarrhea.

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