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Original Article

Molecular Identification of Virulence Genes *Salmonella Enterica* Isolated of Animal and Human Diarrheal

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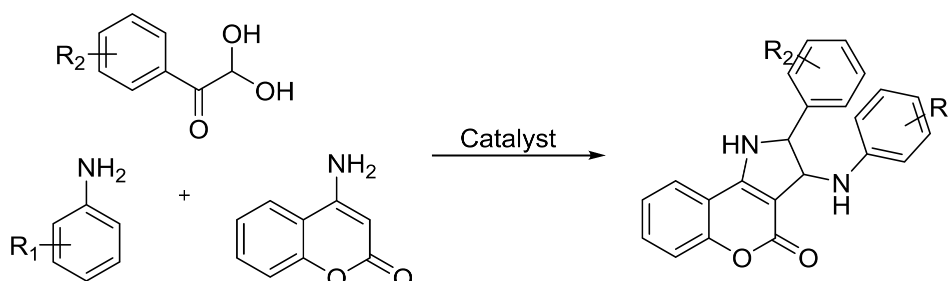
ABSTRACT

Salmonella enterica is the leading cause of food poisoning in some countries. Salmonella species are the most prevalent causes of foodborne illness in humans and animals. *S. enterica* virulence genes were identified using PCR on 400 animal and human samples with specified primers. 6.25% percent of samples were examined by bacteriology and *16S rRNA*. 12 (6%) animal and 13 (6.5%) human samples had *S.enterica.* All of isolates had invasive gene *invA,* Salmonella enterotoxin gene *stn*, and plasmid-encoded fimbriae *pefA*. Based on the results, the *invA* and *stn* virulence genes are stable in *S. enterica* cause diarrhea, and could be used on their own as a gene marker to quickly find virulent strains of *S. enterica,* while the *pefA* gene was only found in isolates from a few known sources. Testing for virulence genes with PCR revealed that the *invA* and *stn* genes are crucial for the serovars of *S. enterica* to be virulent in the host, demonstrating how harmful it is to feed these zoonotic organisms to people. *S. enterica* isolates appeared to be easily discovered using PCR assays that included the invA and stn virulence genes. In PCR tests, using the invA, pef, and stn virulence genes appears to be a quick, accurate, and precise way to distinguish S. enterica isolates.

GRAPHICALABSTRACT



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Introduction

Salmonella species are the most common ones that make people and animals sick after eating. About 93.8 million people get gastroenteritis every year because of non-typhoidal *Salmonella.* This is a problem for public health around the world [1]. Salmonella enterica are a major public health risk. These pathogens exploit multiple virulence factors to cause human infections [2]. *Salmonella enterica Typhimurium* (ST) causes the majority of instances of food poisoning in several nations. It is classified as a 2012 zoonotic not species-specific bacteria that has the potential to spread disease to both humans and animals and although the majority of infections caused by this microbe merely result in self-limiting gastroenteritis, it can also cause more severe conditions [3]. When this bacterium reaches the stomach, it should deal with the acidity of the environment. Therefore, its acid tolerance response (ATR) is engaged, which retains an intracellular pH that is higher than the outside pH, allowing it to live [4]. *Salmonella spp.* penetrates the mucus layer that is already presented in the intestinal wall and its ability to stick to the epithelium, which is where the infection will occur [5]. The interaction of Salmonella species with the epithelium results in the development of a clinical syndrome of diarrhea, loss of electrolytes, and inflammation of the digestive tract are the hallmarks of this condition [6]. A vast number of genes that are found on the chromosome of the bacteria itself are responsible for encoding the virulence factors, the so-called housekeeping genes, and confer certain basic characteristics on bacteria of the same family. Genes pathogenicity islands these genes can also be found in the elements of genetic information that are mobile, such as transposons, plasmids, and bacteriophages [7]. Several virulence genes in *S. enterica* encoded to products that help the organism to express its virulence, in the host such as *Inv, sef, and pef* which are considered to virulence genes for pathogen attachment and invasion in the host tissues [8]. Fimbriae are located on the cell surface of certain bacteria that have been proven to play a significant part in the creation of colonization, and the initial attack on the host [9]. The salmonella enterotoxin gene (stn) is commonly found in many different Salmonella serotypes found in all strains of Salmonella which the *stn* gene was chosen due to the *Salmonella enterica serovars* having a high level of specificity and conservation [10]. Salmonella plays a role in the occurrence of diarrhea and fever through because of it possess various virulence factors, such as cilia, flagella, and biofilmæ that help bacteria adhere to the epithelial cells of the intestines, resistance to antibiotics, and escape from phagocytosis, as well as the possession of heat labile toxins, which helps in the diarrhea occurrence [11]. With studies resulted in the development of polymerase chain reaction (PCR) techniques targeting four potential virulence genes demonstrated that *Salmonella spp.* isolated from animal and human diarrheal. Therefore, to use genetics to tell the difference between animal and human Salmonella, most of the genes this study at came from animal and humans *invA, 16SrRNA, stn, and pefA genes*. The aims of study were to detect virulence genes of *Salmonella enterica* such as Invasion gene *(invA)*, Enterotoxin gene *(stn)*, and plasmid encoded fimbria gene *pefA*.

Materials and Methods

Sample collection

Four hundred fecal samples were collected from animals with suffering diarrhea from farms and veterinary medicine and human suffering diarrhea in Mohammed Al-Mosawi and Bnti-Huda Hospitals in Thi-Qar province, south of Iraq during the period from December 2021 to July 2022.

Isolation and identification of salmonella spp.

The samples were then cultured on several selective media, for identification of *salmonella* colonies, and then incubation at 37 °C for 24 hours. The growth isolates were put through various biochemical tests, and finally confirmed using Api20-E system Colonies that showed biochemical characteristics similar to that of *Salmonella* spp. were tested by API20-E system

and the confirmation was identified by PCR with *16S rRNA.*

Specific primers sequence used for PCR amplification

Primers utilized for the detection of the particular sequence of the *1Ss rRNA* gene ribosomal genes of *Salmonella* spp. and virulence factor genes include *invA* gene encoding proteins, *(stn) gene*, and *(PefA) gene*. The NCBI Gen Bank was utilized in the detection of these primers, which were particular to this investigation and provided from IDT (Canada), as presented in Table 1.

Polymereas chain reaction PCR

Using the Geneaid Genomic DNA Purification Kit (Turkey) and adhering to the procedures outlined by the manufacturer, genomic DNA was extracted from 25 different Salmonella isolates. After this was completed, the bacterial culture was injected in 10 milliliters of nutritional broth medium and incubated at 37 °C overnight in shaking incubator. The other components were added to the reaction mixture according to the company's instructions as in the following (PCR master mix 25 µL, DNA template 5 µL, Forward Primer 10 pmol 3 µL, Reverse Primer 10 pmol 3 µL, and Nuclease free water 14 µL) which can be finally visualized after agarose gel electrophoresis was prepared according to [14]. Then, the agarose removed from the tank and visualized with the aid of the UV transillluminator and photographed.

Results and Discussion

(25) pre-identified *Salmonella enterica* serovars isolates were identified at subspecies level into 12 (6%) isolates from animals and 13 (6.5%) isolates from humans. Screening of *Salmonella* spp. isolates from diarrhea sample in this study genus-specific *16S rRNA* gene (polymerase chain reaction) analysis was done on the bacteria. A total of 12 (6%) from animals and 13 (6.5%) from humans *S. enterica*. among these in the first set of PCR which run *16S rRNA* 1500bp genes resulted positive amplifications in 25 (100%) isolates (Figure 1).

Table 1: Specific primers used for the detection specific sequence of *salmonella enterica*

Gene	Primer Sequences (5'-3')		Size(bp)	Reference
(invA)	F	ACGTTTCCTGCGGTACTGTT	621	[12]
	R	CGTCATGATATTCCGCCCCA		
16sRNA	F	AGAGTTTGATCCTGGCTCAG	1500	[13]
	R	GGTTACCTTGTTACGACTT		
(PefA)	F	CAGGGTTGTGCAAATCTGGC	273	[8]
	R	TGCGAAAGATGCCACAGACT		
(stn) gene	F	TCTCGTCCATCAAAGGGCTG	549	[8]
	R	TTTTCACCTTAATCGCGCCG		

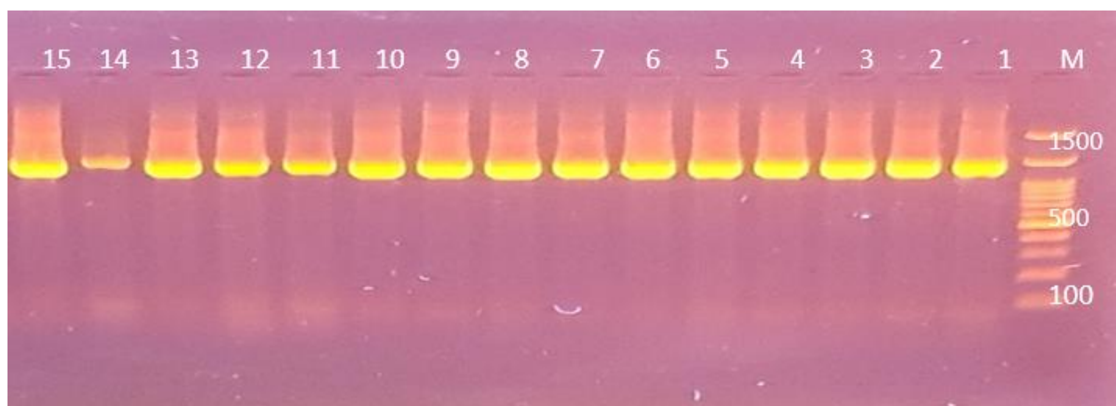

Figure 1: PCR products of the *16S rRNA* gene of *S. enterica*. The size of the PCR product is 1500 bp M: Marker DNA ladder (100-2000)

In developing countries, Salmonella is considered to be the most significant contributor to the phenomenon in question food-borne diseases and the rate of mortality and morbidly [1]. The results of *Salmonella* isolates are approximate with many studies performed in several locations in Iraq for the proportions of *Salmonella* isolation [15, 16]. The current study agrees with another study done among 400 stool samples collected from children only 20 patients were positive (5%) in Thi-Qar [17] and agrees with study [18] which observe that out of 100 samples were positive to *Salmonella* with 16% prevalence. In addition, the results are in agreement with [19] in Basra.

It is probable that the huge variation in Salmonella isolation rates is due to the fact that researchers looked at the pathogen at different periods and geographic locations. This is due to the fact that the manifestations of the disease change from location to location and throughout the history course, depending on the local meteorology, geography, and poor hygiene, in addition to the environmental pollution in addition to the incorrect use of medicines are the major factors for Salmonellosis infection. The inadequate sanitary conditions are also a contributing factor.

Salmonella virulence is connected to a lot of different virulence factors, although in this particular study, just a handful of those components were evaluated using PCR. In the initial set of PCR tests, which were performed on *invA* (621 bp), *stn* (549bp), and *pefA* (273 bp) genes resulted in positive amplifications in 25 (100%), 25(100%) and 12 (48%) isolates, respectively (Figures 2, 3, and 4).

Figure 2: PCR products of the *invA gene* of *S. enterica*. The size of the PCR product is 621bp M: Marker DNA ladder (100-1500)

Figure 3: PCR products of the *stn* gene of *S. enterica*. The size of the PCR product is 549 bp M: Marker DNA ladder (100-1200)

Figure 4: PCR products of the *pefA* gene of *S. enterica*. The size of the PCR 273 bp M: Marker DNA ladder (100-1200)

The PCR detection of *invA*, *16S rRNA*, *snt, and pefA genes* among the isolates of *S. enterica* signified the highest risk by these zoonotic bacteria into humans. Salmonella's *invA* gene participates in the adherence and the pathogen invasion to the host as an *inv* gene or aid in the pathogen's survival within the host [20]. The *invA gene* encodes of membrane proteins required for bacterial invasion of the host epithelial cells. This gene is on pathogenicity island I, also known as the Salmonella Pathogenicity Island (SPI); the DNA area is associated with the pathogenicity of *Salmonella enterica* and is possessed by all serotypes [21]. As previously reported, this gene is a suitable target for the Salmonella detection in various species of organism. The widespread presence of the virulence gene *invA* in Salmonella species poses a risk to the public health and frequently leads to losses. Using polymerase chain reaction (PCR), several researchers investigated the possibility of the *invA* gene identification in *S. enterica* strains that originated from clinical fecal or dietary samples [22]. This study found that 25 (100%) of *Salmonella* isolates contained the *invA* gene. Many studies showed in this gene in *Salmonella*, such as [23, 24] an agreement with [20, 25-27]. *InvA* gene was used as a golden marker in the genetic diagnosis of Salmonella species because reported that invA is found only in Salmonella species [28].

Therefore, they hypothesized that *stn* is a Salmonella virulence factor and is accountable for Salmonella's enterotoxicity [29]. In present study, the percentage positive all samples *salmonella enterica* is 100% in Agreement [30, 31]. In addition, the present study is in agreement with the study [11].

The results are consistent with the findings of another research indicating the *stn* gene presence in all Salmonella isolates used in those investigations, regardless of the strain100% of the strains and sources of isolation [32, 33]. This gene is an important gene for the Salmonella bacteria. Salmonella bacteria's expression of the specific virulence factors can be influenced by environmental change. It is believed that unique environmental conditions contributed to the presence of the *stn* gene in all isolates from the infected patient. It is interesting to note that the *stn* gene is exclusive to Salmonella [34, 35]. Previous research demonstrated that the *stn* gene is widely distributed among several Salmonella serovars isolated from numerous of hosts, including humans, cattle, and birds [36, 37]. Several investigations, both *in vitro* and *in vivo* have demonstrated that fimbriae play an important role and are involved in several pathogenic processes: Adherence to particular epithelial cells (such as type I fimbriae, curli fimbriae, *Pef, Lpf,* and *Std* [38]. In the present study, the percentage positive 12/25 (48%) arrange between in 7/12 (58.3%) in animals and 5/13 (38.4%) in humans. This study is in agreement with [23] as percentage 18 (51.42%). In a study that was similar the researchers looked at the distribution of the *pef* gene among 95

Salmonella isolates that had originated from samples of poultry, pigs, cattle, and people all 95 isolates (100%) were found to *pef* gene and 36 (37.89%) isolates were positive for *pefA* [7]. Another investigation used 23 *S. enterica* isolates from pig and chicken feces, and 11 isolates were positive for *pefA* [22]. Because the *pefA* gene was originally plasmid-based, it is possible that some salmonellae do not have it because the plasmid is where the gene started. Plasmids are exclusively found in selecting few serotypes of Salmonella species and strains [39, 40].

Conclusion

Molecular approaches are needed to analyze and identify these pathogens which have high percentages of virulence factor genes in *Salmonella. Enterica* makes these pathogens more hazardous**.** Some virulence genes of *S. enterica* increase their pathogenicity especially *invA, stn*, and *pefA*.

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Authors' contributions

All of the authors participated in the data analysis, drafting, and editing of the publication and accepted full responsibility for all elements of this work.

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