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Original Article

Molecular Detection of Some Virulence Genes in *Salmonella* Species Isolated from Clinical Samples in Iraq

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Abstract

Salmonella species (spp.) are a major source of diarrheal diseases everywhere and one of the most dangerous foodborne bacteria. The present study aimed to detect the occurrence of the most important virulence genes in *Salmonella enterica* (*S. enterica*) among bacteria isolated from stool in Baghdad hospitals, Iraq. In total, 50 swab stool samples were collected from patients suffering from food poisoning, attending to different hospitals in Baghdad. The isolates were identified using morphological tests and were confirmed by the Vitek-2 system (BioMe´rieux, France). A genomic DNA kit (Qiagen, Germany) was utilized to extract DNA from the isolates. Molecular detection of five virulence genes, including *invA*, *papC*, *spvC*, *stn*, and *fimH*, was performed using Polymerase Chain Reaction (PCR). Out of 50 swab samples, 40% (20 samples) were confirmed as *S. enterica*. Moreover, the prevalence of virulence genes determined by the PCR demonstrated that all 20 *S. enterica* isolates carried at least one gene from those associated with biofilm formation. The *invA*, *stn*, and *fimH* were the most predominant genes existing in all 20 *S. enterica* isolates. The prevalence of *papC* and *spvC* virulence genes was 75% (15 out of 20) and 65% (13 out of 20), respectively. The current data support the occurrence of *Salmonella* spp. exhibiting a broad range of virulence genes in stool samples from patients who had food poisoning, which indeed makes these bacteria a significant threat to public health.

Keywords: Foodborne illnesses, PCR, *Salmonella*, Virulence genes

1. Introduction

Salmonella species (spp.) are a major source of diarrheal diseases everywhere and one of the most dangerous foodborne bacteria (1). They are gram-negative facultative anaerobe pathogens and the second most known predominant foodborne bacteria due to their broad prevalence besides their efficiency in making extensive infections (2). *Salmonella* settles down the intestine of many animals, such as dogs, cats, and birds (3). In poultry, the bacterium is considered an effective carrier facilitating the dissemination of different *Salmonella* serovars to humans through eating contaminated meals (4). Pigs and poultry are among the main animals accountable for *Salmonella* dissemination to people (5).

Salmonella outbreaks are indeed a leading factor for illness and death in cows and sub-clinically afflicted animals. As a result, cows serve as a significant reservoir for human illnesses (6). It has been established that the occurrence of virulence genes, which play a crucial influence in the incidence of serious invasions, contributes to the pathogenicity of bacteria inside the host organism (7). *Salmonella*'s distinct virulence genes, including *inv*, *spv*, *stn*, *fim*, *pap*, *pef*, *viz*, and *sop*, are associated with adhesion and the invasion of host cells. They are also involved in bacterial demonstration and survival (8).

The *invA* and *stn* gene sequencings have a central role in the identification of *Salmonella*, discovering new

species, and giving a wide scope of analysis. Gene sequencing is a rapid technique for bacterial identification of unknown phenotypes (9). Although *Enterobacteriaceae* have emerged as the most questionable pathogens of critical health care interest, little is known about their virulence genes, which contribute to their pathogenesis (2).

Recently, advanced molecular techniques have been developed for the detection of foodborne microbes depending on nucleic acid amplification due to their rapidity, specificity, and sensitivity (10). In light of these facts, the current study aimed to detect the occurrence of the most important virulence genes in *Salmonella enterica* (*S. enterica*) among bacteria isolated from stool in different hospitals in Baghdad.

2. Materials and Methods

2.1. Isolation and Identification of *Salmonella* Isolates

A total of 50 swab stool samples were collected between June 2019 and March 2020 from patients suffering from food poisoning, who attended to different hospitals (Medical City hospital, Al-Zahra hospital, Al-Yarmouk hospital, and Ibn Balady hospital) in Baghdad, Iraq. The isolates were cultured on the Xylose lysine deoxycholate (XLD) agar, Hektoen enteric (HE) agar, and *Salmonella-Shigella* (S-S) agar (Himedia, India). Afterward, they were incubated at 37˚C for 24 h and then, identified using the biochemical Vitek-2 system (BioMe´rieux, France).

2.2. Extraction of DNA

To extract DNA from the isolates, a genomic DNA kit (Qiagen, Germany) was applied, according to the manufacturer's recommendations in the leaflet. Briefly, a volume of 200 µl of each isolate was suspended equal to 0.5 McFarland standard, 10 µl of proteinase K was added to it with 200 µl of lysis solution, and it was incubated for 10 min at 56˚C. After the incubation period, 200 µl of absolute ethanol was added. Afterward, the samples were washed three times and centrifuged. The elution solution (with 100 µl) was then added to elute the DNA. The extracted DNAs were preserved at –80˚C for further usage as a template in the polymerase chain reaction (PCR).

2.3. Molecular Detection of Virulence Genes

Specific primer sequences of five virulence genes, including *invA*, *papC*, *spvC*, *stn*, and *fimH*, were amplified by the PCR. Table 1 shows primer sequences and product sizes. Primers were utilized for the PCR amplification using a 25 µl volume for the reaction. The PCR mixture contained 12.5 µl of Master mix, 1 µl (0.6 pmol) of F and R each primer, and 3 µl of DNA template. Finally, the volume was completed by adding 25 µl of nuclease-free water (Promega, USA). The amplification reaction was performed in a TechNet-500 thermocycler (USA). The PCR products were analyzed using electrophoresis with 1% agarose gel stained with 0.5 µg/ml ethidium bromide at 7 V/Cm for 90 min and DNA ladder (100 bp, Promega, USA). The gel was photographed under a UV transilluminator.

2.4. Statistical Analysis

The data were analyzed using the Graph Pad Prism 8 software for each *S. enterica*. in biological test replication. The probability level was set at $P \leq 0.05$ to identify significant differences.

Table 1. List of primers or Polymerase Chain Reaction amplification

Gene	Sequence	Product size (bp)	Annealing temperature (˚C)	Reference
invA	GTGAAATTATCGCCACGTTCGGGCAA TCATCGCACCGTCAAAGGAACC	284	55	(11)
papC	GACGGCTGTACTGCAGGGTGTGGCG ATATCCTTTCTGCAGGGATGCAATA	328	63	(12)
spvC	ACTCCTTGCACAACCAAATGCGGA TGTCTTCTGCATTTCGCCACCATCA	571	53	(13)
stn	TTG TGT CGC TAT CAC TGG CAA CC ATT CGT AAC CCG CTC TCG TCC	617	59	(7)
fimH	TGC AGA ACG GAT AAG CCG TGG GCA GTC ACC TGC CCT CCG GTA	508	63	(14)

3. Results

3.1. Isolation and Identification

The phenotypic results of *Salmonella* spp. on different cultured media, including the XLD agar, HE agar, and S-S agar, showed that out of 50 swab samples, 40% (20 samples) were confirmed as *S. enterica* followed by the biochemical Vitek-2 system (BioMe´rieux, France). Figure 1 shows the morphological properties of the tested isolates on different cultured media.

3.2. Prevalence of Virulence Genes

As for the detection of virulence genes, the PCR demonstrated that all 20 *S. enterica* isolates carried at least one gene from those associated with biofilm formation. The *invA*, *stn*, and *fimH* were the most predominant genes existing in all 20 *S. enterica* isolates. The prevalence of *papC* and *spvC* virulence genes was 75% (15 out of 20) and 65% (13 out of 20), respectively. Table 2 shows the molecular characterization of the studied virulence genes in *Salmonella* isolates. Furthermore, figure 2 depicts the amplified studied virulence genes in *Salmonella* isolates by using agarose gel electrophoresis.

Figure1. Identification of *Salmonella* isolates. (**A**) *Salmonella enterica* on XLD agar. (**B**) *Salmonella enterica* on HE agar. (**C**) *Salmonella enterica* on S-S agar

Table 2. Molecular characterization of some virulence genes in *Salmonella* isolates

Tested virulence genes	Source of isolates	No. of examined isolates	No. of positive isolates	Percentage %
invA	Stool	20	20	100%
stn	Stool	20	20	100%
fimH	Stool	20	20	100%
papC	Stool	20	15	75%
spvC	Stool	20	13	65%

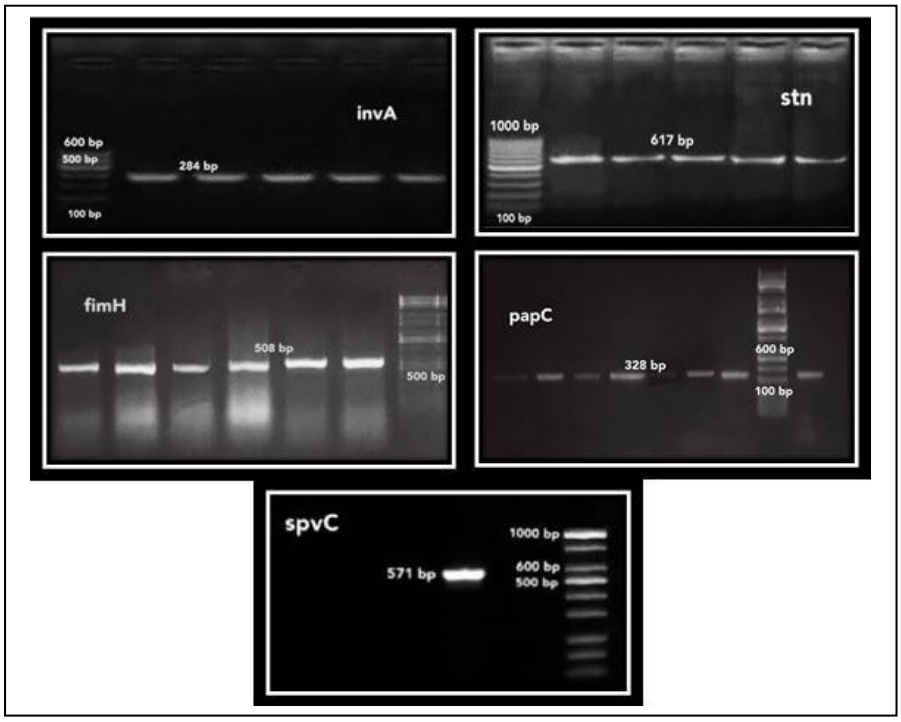

Figure 2. Gel electrophoresis for *invA*, *stn*, *fimH*, *papC*, and *spvC* genes

4. Discussion

Salmonella is an opportunistic pathogen that is commonly implicated in foodborne disease outbreaks. Its ubiquitous distribution poses a significant threat to public health (15). In this study, 20 out of 50 stool swab samples were positive for *S. enterica*, accounting for 20% of the total. These findings were consistent with previous research findings, including (8, 16, 17). Traditional strategies are widely accepted as basic techniques for detecting foodborne bacterial diseases. They are widely utilized due to their ease of use and safety, and they can provide information on the type, size, and behavior of food microorganisms (18, 19). Unfortunately, however, these techniques require plenty of personnel and takes a couple of days to perform; therefore, they are frequently inefficient.

According to table 2, *S. enterica* isolates exhibited 100% of virulence genes (*invA*, *stn*, and *fimH*), which is the same as the previous research findings done by (8, 20). The *invA* gene has been proposed as an indicator for the molecular analysis of *Salmonella* serotypes identification whereas the *stn* gene is in charge of enterotoxin generation (20). According to the findings of a previous study carried out by Uchiya, Kamimura (21), *fimH* is indeed an adhesive protein that is essential in bacterial adherence to host cell receptors throughout the host tissue infarction (22).

The *stn* gene seems critical in determining the virulence and intensity of invasion inside particular hosts.

Since it is present in all serotypes of *Salmonella* spp., *st n* is employed for the

detection and characterization. It is also critical due to causing *Salmonella* enterotoxicity (23).

According to Robertson, Yoshida (24), the value of such evidence is a concern for public healthcare and dietary quality organizations, since erroneous or absent metadata significantly diminishes the value of genomic information. The data gathered from multiple studies might be used to create better techniques for eliminating pathogens inside food webs, thereby decreasing foodborne illnesses in the animal-food cycle and maintaining a successful program monitoring (25). The virulence of the acquired *Salmonella* isolates is

correlated to a set of genes that seem essential for the entrance, colonization, and dissemination inside the host body.

A study by Mezal, Sabol (26) indicated that all isolates were positive for the majority of virulence genes present in *S. enterica*, which is consistent with the findings of the present study (27, 28). *Salmonella* represents the second most common foodborne illness in the United States and Europe (29). As a result, awareness of the development of infections is becoming extremely essential to study the occurrence of *Salmonella* in the environment and its virulence patterns. According to the best of the researchers' knowledge, there is little information about the molecular detection of virulence genes in *S. enterica* isolated from clinical samples in Iraq. Therefore, further studies must understand the virulence genes of these bacterial isolates using rapid molecular diagnostic methods fundamental for operative clinical management.

The current data support the occurrence of *Salmonella* spp. exhibiting a broad range of virulence genes in stool samples from patients who had food poisoning, which indeed makes these bacteria a significant threat to public health. The risk of these dangerous pathogens being transmitted to people necessitates continuous supervision of the supply chain and modified food production, as well as special attention to antimicrobial therapy. More investigation is necessary to offer precise information regarding the prevalence rate of *Salmonella* in food and feces, as well as sanitary approaches to minimize the spread of infection.

Authors' Contribution

Study concept and design: H. F. A.
Acquisition of data: H. F. A.
Analysis and interpretation of data: H. F. A.
Drafting of the manuscript: H. F. A.
Critical revision of the manuscript for important intellectual content: H. F. A.
Statistical analysis: H. F. A.
Administrative, technical, and material support: H. F. A.

Ethics

The present study was approved by the Ethics Committees of the Iraqi Ministry of Health and the Mustansiriyah University.

Conflict of Interest

The authors declare that they have no conflict of interest.

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