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Prevelance of *Stn* and *FimH* Genes in *Salmonella enterica* Isolated from Patients in Thi-Qar Governorate

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Abstract: *Salmonella* spp. are a significant cause of diarrheal illnesses worldwide and are among the most hazardous bacteria transmitted through food. The aime of study detect the existence of crucial virulence genes in *Salmonella enterica (S. enterica*) bacteria obtained from stool samples gathered from Thi-Qar hospitals in Iraq. A total of 100 stool samples were obtained from patients with diarrhea symptom at several hospitals in Thi-Qar. The isolates were initially identified through morphological characteristic on XLD media and later confirmed using the Vitek-2 method. The DNA was extracted from (20) isolates using a genomic DNA kit. The PCR technique was utilized for detection of *Salmonella enterica* by using *invA* .Other virulence genes suvh as *FimH,* and S*tn* at the molecular level. Out of the 100 swab samples , 20% (20 samples) were positively identified as *S. enterica*. The occurrence of *invA* , *Stn* and *FimH* virulence genes was percentage (100%) and in (75%) of the samples (15 out of 20) and (20%) of the samples (4 out of 20), respectively . The available findings confirm the presence of *Salmonella enterica*. with a wide array of virulence genes in stool samples, thus establishing these bacteria as a substantial public health risk.

Keywords: *Salmonella enterica, stn*,*invA* ,*fimH*,virulence genes, pcr.

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Introduction

Salmonella species are significant contributors to diarrheal illnesses globally and represent one of the most perilous types of bacteria transmitted through food (1). *Salmonella* is a prevalent source of bacterial infections. Among the most significant infections that can be transmitted through the intake of meat and meat products is considered (2). These pathogens are facultative anaerobes that belong to the gram-negative group. They are the second most commonly found bacteria in foodborne illnesses. They are noted for their ability to cause widespread infections. *Salmonella* establishes itself in the digestive system of different animals, such as dogs, cats, and birds (3). Within the poultry industry, the bacterium is recognized as a proficient vector that aids within the transmission of several *Salmonella* serovars to humans by consumption of contaminated food (4). Poultry and Pigs are the primary animals responsible for the transmission of *Salmonella* to humans (5). *Salmonella* outbreaks are a significant cause of disease and mortality in cows and animals that are

not showing obvious signs of infection. Consequently, cows act as a substantial reservoir for human diseases (6). Studies have confirmed that the presence of virulence genes, which have a significant impact on prevalence severe infections, provides to the ability of bacteria to cause disease within the host organism(7). The virulence genes of *Salmonella*, such as inv, *spv*, *stn*, *viz*, *sop*, *fim* , and *pef*, are specifically linked to the Capability of the bacteria to Attach to and infiltrate host cells. Additionally, they participate in the exhibition and endurance of bacteria (8). Despite Enterobacteriaceae being recognized as the most concerning pathogens in critical healthcare settings, there is less knowledge regarding their virulence genes that play a role in their ability to cause disease (9).

In recent times, there have been advancements in molecular approaches used to identify foodborne bacteria. These techniques rely on nucleic acid amplification and offer advantages such as speed, specificity, and sensitivity (10). The objective of this study was to detect the existence of some virulence genes in *Salmonella enterica* (*S. enterica*) bacteria that were obtained from stool samples collected from different patients seeking medical device some hospitals in Thi-Qar.

Materials and methods

Collection of stool sample

One hundred stool samples were obtained from patients in the stages of all ages and both sexes suffering from watery diarrhea or with (mucus, pus, little blood or no) after cultured on XLD media as figure (1). This sample were admitted in three hospitals of Thi-Qar (from October 2023 to February 2024.

Extraction of DNA

The DNA from the *Salmonella enterica* isolates was obtained utilizing The process of extraction. The DNA extraction from the isolates was conducted utilizing a genomic DNA kit produced by Bioneer, a Korean-based business. The concentration and purity of the isolated DNA were measured utilizing the Qubit 4 system. and subsequently confirmed using gel electrophoresis.

Molecular Detection of some Virulence Genes

The PCR techinque was used for further conformation of *Salmonella enterica* by use *invA* gene and some virulence genes (*FimH,Stn*) genes ,specific primer sequences of virulence genes, specifically *invA*, *Stn*, and *FimH*, are provided Table (1) displays the primer sequences and the product of sizes and Table (2) showed Specify the protocol for PCR amplification for each gene in the study. PCR amplification was performed using primers in a reaction volume of 25µl (11). The PCR mixture comprised the experiment requires 12.5 µl of Master mix, 1.5 µl of each F and R primer, and 4 µl of DNA template. Ultimately, The volume was completed by adding 25 µl of nuclease-free water. The amplification process was performed using a thermocycler apparatus. The PCR results were examined via electrophoresis on a 2% agarose gel, which was dyed with 1.5 µg/ml of red safe dye. The electrophoresis was conducted at a voltage of 80V for a duration of 80 minutes. A DNA ladder measuring 100 base pairs. The gel was vituated see photographe by using gel documentation system.

Table (1): Primer sequences and size which used in this study for identification of genes.

Target gene	Nucleotide sequence (5'—3')	Size Product bp	The Reference
invA	F:GTGAAATTATCGCCACGTTCGGGCAA	284	(12)
	R:TCA TCG CAC CGT CAA AGG AAC C		
Stn	F:CTT TGG TCG TAA AAT AAG GCG	260	(12)
	R:TGC CCA AAG CAG AGA GAT TC		
FimH	F:TGC AGA ACG GAT AAG CCG TGG	508	(13)
	R:GCA GTC ACC TGC CCT CCG GTA		

Table (2): Specify the protocol For each gene in this study of PCR amplification.

Steps	Cycles	Genes amplification conditions			
		invA	***Stn***	***FimH***	**Time**
Initial denaturation	1 cycle	94 ºC	94ºC	94ºC	5 mins
Denaturation	35 cycle	94 ºC	94ºC	94ºC	30 sec
Annealing		57 ºC	49ºC	58ºC	45 sec
Extension		72 ºC	72ºC	72ºC	45 sec
Final extension	1 cycle	72 ºC	72ºC	72ºC	7 mins

Results and discussion

Isolation and identification of *Salmonella* enterica

Stool samples were collected under the aseptic condition in a tube (5-15 ml). The specimens were immediately transported to buffer peptone water and incubated at 37C˚ for 24h then cultured on tetrathionate broth and then incubated aerobically at 37 C˚ for 24h. after this culturing on XLD agar was incubated aerobically at 37C˚ for 24h as shown in (Figure1).

Colony identification on these medium was accomplished using biochemical tests. The biochemical test results were validated using the VITEK-2 system from BioMerieux, France.

Detection of *(invA, Stn, FimH)* genes by PCR techniqne

Salmonella is an pathogenic bacteria frequently associated with outbreaks of foodborne illnesses. The widespread presence of it presents a substantial risk to the well-being of the general population (14). Several bacterial species, such as *Salmonella enterica*, are responsible for causing diarrhea. Diarrhea is characterized by the excretion of liquid feces on at least three occasions within a 24-hour period (15). Out of the 100 stool swab samples analyzed in this study, Out of the total, (20%) were found to be positive for *S. enterica*, with a total of 20 individuals testing positive. The findings were nearly consistent with previous research (16) where the researcher found 14 *Salmonella enterica* isolates were isolated from diarrheal children in Thi-Qar province. Conventional methods are generally acknowledged as fundamental approaches for identifying foodborne bacterial illnesses. They are widely used because of their ease of use and safety, and they can provide information about the classification, size, and behavior of food microbes (17,18). Table (3) which clarify the distribution of (*invA, FimH, Stn*) genes in *Salmonella* indicates that *S. enterica* isolates displayed a 100% presence of the *invA* virulence gene, consistent with prior study conducted by Ahmad and Mustafa (19) the investigation indicated above verified the existence of 37 isolates, all of which tested positive for the *invA* gene. This agreement is

necessary since the presence of this gene is required in all isolates, The *invA* gene is proposed as a molecular marker for the research of *Salmonella* serotype identification (16), whereas the *Stn* gene is responsible for the synthesis of enterotoxin,*Stn* gene is essential in establishing the level of pathogenicity and extent of infiltration inside particular hosts. *Stn* is utilized for the identification and characterization of *Salmonella* spp. due to its presence in the majority of serotypes. It is also crucial since it leads to *Salmonella* enterotoxicity (20), the *Stn* present in (15) out of (20) isolates Approximately (75%) of isolates this result disagreement with Nikiema *et al* (8) may be due to the difference in the study area,and the *FimH* gene presence in (4) out of (20) isolates (20%) while, Abbas conducted study in the city of Baghdad and the number of isolates was (20). All isolates were positive for the *fimH* gene (21). According to the findings of a previous inquiry carried out by Uchiya and Kamimura (22),the *fimH* gene plays a crucial role in pathogenicity by contributing many virulent features, including the adherence of bacteria to the epithelial tissue (23) Caution should be exercised when comparing our results with those of other regional studies due to variations in sample size, research length, and laboratory testing procedures,this may be due to the difference in time and place of study. These findings align with the identification of the majority of other categories of bacteria that are spread by water and food, such as *cholera* bacteria (24). Robertson and Yoshida (25), the public healthcare and dietary quality organizations are concerned about the value of evidence in genomics. They emphasize that inaccurate or missing metadata greatly reduces the usefulness of genomic material. The data collected from various research can be utilized to develop more effective methods for eradicating pathogens within food chains, consequently reducing instances of foodborne diseases in the animal-food cycle and ensuring the efficacy of the monitoring program (26). *Salmonella* ranks as the second most widespread foodborne illness in both the United States and Europe(27). Whereas. In Baghdad, central Iraq, non-typhoidal *Salmonella* was the second most frequently reported cause of diarrhoea in children under age 5 (28) Consequently, it is crucial to research the occurrence of the presence and distribution of *Salmonella* in the environment and its patterns of virulence in order to understand the genesis of infections. Based on the researchers' understanding, there is limited data on the molecular identification of virulence genes in *Salmonella enterica* collected from clinical samples in Iraq. Hence, it is nesessary to conduct additional research to comprehend the virulence genes of these bacterial isolates by the use of molecular diagnostic techniques. Continuous oversight of the supply chain and changed food production, together with careful consideration of antibiotic therapy, is necessary due to the possible transfer of these dangerous germs to humans. Additionally, Figures (2,3,4) showen the bands of genes on gel electrophoresis *invA,stn* and *fimH* Respectively.

Table (3):The distribution of (*invA,stn,fimH*) genes in *Salmonella enterica* isolates.

Virulence genes	Source of isolates	No. of analysis isolates	No. of positive isolates	Percentage %
invA	Stool	20	20	100%
Stn	Stool	20	15	75%
FimH	Stool	20	4	20%

Figure (1): The morphology and color of colonies of *Salmonella* culture on XLD showed small smooth, rounded with black center colonies.

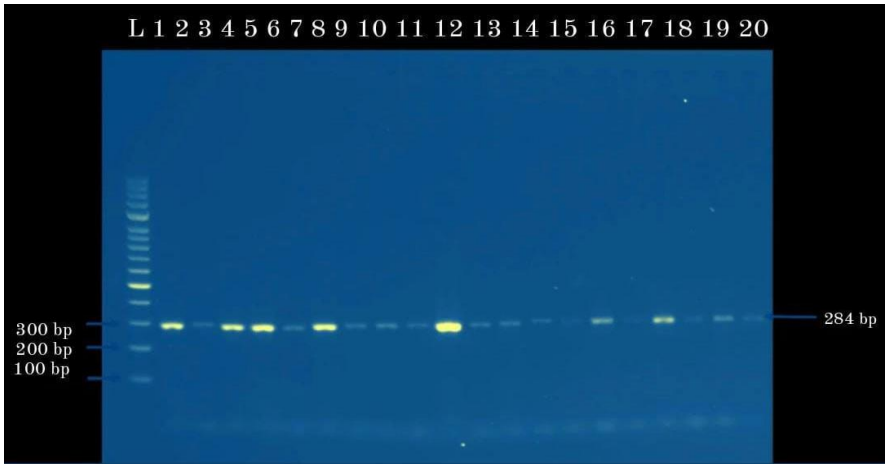

Figure (2): Gel electrophoresis of *invA* gene of *S.enterica* isolates using (2% agarose gel electrophoresis 75% volt for 2 hours).L:1000Pb ladder marke. lame 1-20 resemble PCR product.

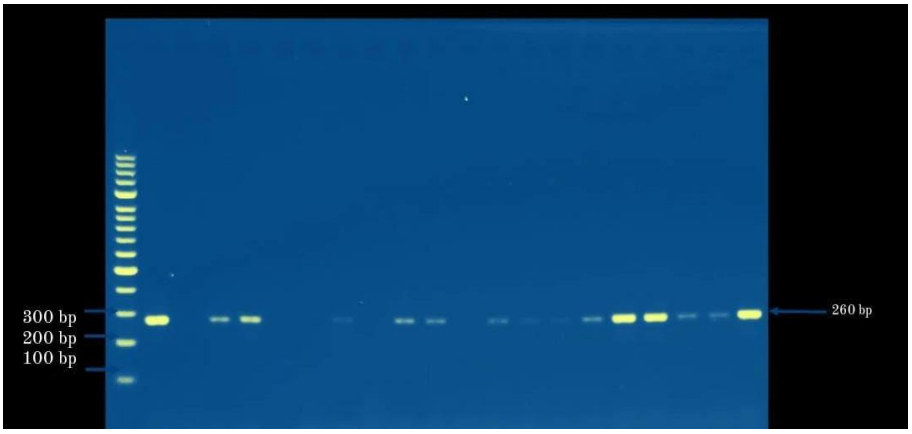

Figure (3): Gel electrophoresis of *Stn* gene of *S.enterica* isolates using (2% agarose gel electrophoresis 75% volt for 2 hours). L:1000Pb ladder marke. lame 1-20 resemble PCR product.

Figure (4):Gel electrophoresis of *FimH* gene of *S.enterica* isolates using (2% agarose gel electrophoresis 75% volt for 2 hours).L:1000Pb ladder marke. lame 1-20 resemble PCR product.

Conclusion

This study conducted that (20%) of diarrheal cases caused by *Salmonella enterica*. All isolates of *S.enterica* possess virulence gene (*invA*). While the other genes such as *Stn* and *FimH* are different in terms of appearance rate.

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