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Molecular Detection of Virulence Genes *fimH* and *csgA* in *Salmonella typhi* Isolated From Stool Samples

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Abstract: Background: Gram-negative *Salmonella typhi*, a bacterium that causes intestinal diseases, including typhoid fever, as a consequence of ingesting foods and drinks contaminated by this bacterium. *Salmonella typhi* uses various virulence factors like, cilia and filaments and fibers that are used in the adhesion and invasion of its host cells.

Objective: This study aimed to isolate, diagnose and investigate the extent of *Salmonella typhi* in stool samples of patients suffering from typhoid fever. In addition, to the identification of virulence genes *fimH* and *csgA*, which provide the ability for *Salmonella typhi* to adhere and help in the formation of biofilms.

Methodology: 250 stool samples were collected in plastic containers from patients visiting hospitals in Al- Diwaniyah city who were suffering from typhoid fever. The samples were then transferred to the laboratory and cultured in a mediums supported the growth of Salmonella. The diagnosis was made using a range of diagnostic and biochemical tests, in addition to Vitek system and serological tests. After

the extraction of DNA, the virulence genes *fimH* and *csgA* of *Salmonella typhi* isolates were studies using PCR techniques.

Results: According to the results showed that 176 (69.6%) stool samples showed a positive result for growth on culture media used in the study, while 76 (30.4%) showed a negative result for culture. and out of 174 positive stool samples of typhoid patients, 20 (11.4%) stool samples contained *Salmonella typhi*. Analysis the results of the PCR method to detect the genes showed that 100% of the isolates contained the *fimH* gene, and 35% of *Salmonella typhi* isolates contained the *csgA* gene.

Conclusions: *Salmonella typhi* was found to be a pathogen because it resulted in infection, it was isolated from 11.4% of typhoid fever patients, *fimH* is the most dominant gene with a 100% rate, and the gene *csgA* has a rate of 35%.

Key Words: *Salmonella typhi*, Typhoid Fever, Stool Samples, Virulence Genes, PCR, *fimH*, *csgA*

Introduction

Salmonella typhi is a bacterial pathogen that is Gram-negative, rod-shaped, anaerobic, non-spore forming and non-enveloped [1]. It is the primary cause of typhoid fever, which is associated with people of low socioeconomic status and lack of general hygiene [2]. Since humans are these bacteria's main host, infection can spread from one person to another, infection can also happen when they are in the environment and come into direct contact with them [3]. Consuming street food, dairy products and ice cream increases risk of infection and transmission, as does not practicing basic personal hygiene and hand washing [4]. The bacteria enter the body through digestive system by consuming food and water contaminated with the feces of patients and carriers of this bacteria [5]. *Salmonella typhi* enters intestinal epithelium after ingestion and then moves on to other organs like the liver, spleen and gallbladder, ten to fourteen days after ingestion infection symptoms appear as headache, fever, intestinal and stomach pain, muscle discomfort, and diarrhea [6]. *Salmonella* have a number of virulence factors, including flagella, cilia, and pathogenic islands, which allow them to cause infection and cause pathogenesis [7]. One of the most important virulence factors that enables these bacteria to cause pathogens is the cilia and hairy appendages that help them to adhere to and colonize the host during infection [8]. Among

the most crucial elements that aid *Salmonella* in colonizing host cells and adhering to different surfaces are cilia and type I filamentary appendages. These appendages are encoded by *fim* operon, which encodes a set of proteins. [9]. The encoded gene *fimH* binds to host cell receptors that have mannose residues, which are crucial for adhering to target cell receptors, one of the key components that aids bacteria in adhering to and invading host tissues is this protein [10]. Another virulence factor of these bacteria is the presence of the curly fibers in the membrane of *Salmonella* that play an important role in the adhesion of bacteria and the initiation of the formation of biofilms by these microbes, these curly fibers consist of two operons, namely, the *csg*D-E-F-G and the *csg*B-A-C operons [11]. These fibers form an integral part of the adhesion mechanism of the microbes and enable them to invade the host cells and evade the host's immune system [12]. In addition, presence of the *csg* operon in *Salmonella* has been linked with the formation of biofilms that help the microbes withstand the effects of antibiotics; therefore, this gene has an impact on the health of the public [13]. The *csgA* gene is a key gene associated with adhesion and biofilm formation, thus enabling bacteria to survive and persist in the host environment [14]. Therefore, due to the importance of this bacterium and the pathogens it causes, this study aimed to isolate and diagnose *Salmonella typhi* from stool samples of patients suffering from typhoid fever and investigating the presence of virulence genes represented by *fimH* and *csgA* genes using polymerase chain reaction technology. These genes allow the organism to adhere and form biofilms, increasing its survival and continuity. Studying spread of these genes in *Salmonella typhi* can help in understanding the mechanisms that help it colonize host cells and perpetuate the infection.

Methodology

Ethical approval

According to the guidelines provided by the ethics committees at Al-Furat Al-Awsat Technical University, general ethics were adhered for the purpose of implementing the study's objectives regarding the collection of stool samples from patients without compromising their health.

Sample collection

250 stool samples were taken from patients who visited Al- Diwaniyah Teaching Hospital and Children's and Maternity Hospital in Al-Diwaniyah City, between (1/8/2024 to 1/12/2024). Sterile, tightly sealed plastic containers were used to collect stool samples, and each patient's details were noted on the container. After that, samples were transferred to the microbiology laboratory for the purpose of performing the culture process.

Cultivation in vitro

Selenite F broth medium, which is a rich medium used to isolate *Salmonella* ssp. from stool samples because it promotes its growth and inhibits growth of other microbial species, was prepared in accordance with the manufacturer's instructions (Himedia), and inoculated with stool samples, after that the tubes were incubated for 24 hours at 37°C [15]. Following that, each stool sample was cultured from Selenite F broth medium by filling the wire loop with the sample and by Streaking on culture media equipped by Oxoid Company. These included Salmonella-Shigella (SS) agar, Xylose Lysine Deoxycholate (XLD) agar, and MacConkey agar, after that the plates were incubated for 24 hours at 37°C [15; 16; 17].

Isolation and diagnosis

Salmonella isolates were isolated and identified based on the morphological characteristics of colonies on the culture media used in the study. The ability of *Salmonella* to ferment lactose was examined using MacConkey agar, and *Salmonella* were distinguished from *Shigella* using Xylose Lysine Deoxycholate (XLD) and Salmonella-Shigella (SS) agar, which are differential media. Microscopic features, such as the shape of bacterial cells under a microscope and examining the result of their reaction with Gram stain were also used in the diagnosis. A range of biochemical

tests were also used, including catalase and oxidase tests, in addition to a set of IMVIC tests such as indole testing, growth on simmon citrate medium, and methyl red voges-Proskauer [15]. Also, a growth test on urea medium and the Triple Sugar Iron test (TSI)\ were carried out [16; 17]. The vitek test kit (Biomerieux) for Gram-negative bacteria was used to confirm the diagnosis and precisely identify *Salmonella* isolates because it is a confirmatory test that yields accurate results, the diagnosis was made in accordance with the kit's manufacturer's instructions.

Serotyping identified of *Salmonella ssp*.

Serological diagnosis was carried out on bacterial isolates exhibiting *Salmonella* traits, by using serum antibodies O and H made by (Oxoid). Young, pure colonies were taken from bacterial isolates, and put on a glass slide, a drop of serum containing antibodies was added to them, they were mixed and the outcome was noted. The absence of a clump or aggregation was regarded as a negative result and the absence of the antigen, whereas the formation of a clump was seen as evidence of the antigen's presence [18] [19].

DNA extraction

The PrestoTM Mini gDNA Bacteria Kit was used to extract DNA from *Salmonella typhi* isolates under investigation and examine the genes in accordance with the manufacturer's instructions. The purity and concentration of extracted DNA were then measured using a nanodrop (USA) device, which measures concentration and purity at 260/280 nm absorption.

Primers

The design of primers used to examine the (*fimH* and *csgA*) genes in *Salmonella typhi* bacterial isolates was based on [20][21] and as indicated in table (1).

Table (1) shows the gene sequences used in the study

Gene Name	Sequences 5'-3'		Size Product
fimH	F	TGCAGAACGGATAAGCCGTGG	508bp
	R	GCAGTCACCTGCCCTCCGGTA	
csgA	F	GCAATCGTATTCTCCGGTAG	418bp
	R	GATGAGCGGTCGCGTTGTTA	

Prepared PreMix Kit

The kit included AccuPower® PCR premix was used to prepare the reaction mixture, and the polymerase chain reaction was prepared according to the manufacturer's instructions (Bioneer, Korea).

Conditions of PCR Thermocycler

Polymerase chain reaction was carried out using the thermal cycler (BioRad/USA) in order to examine the genes (*fimH* and *csgA*), as indicated in table (2).

Table (2) Steps of the PCR reaction for the genes under study

PCR steps / Genes	Initial Denaturation	Denaturation	Annealing	Extension	Cycles	Final extension	Hold
fimH	95 -5 min	95 -30 sec	65 -30 sec	72-30 sec	35	72-5min	4°C
csgA	95 -5 min	95 -30 sec	51- 30 sec	72-30 sec		72-5min	

Electrophoresis

To determine the results of polymerase chain reaction of the (*fimH* and *csgA*) genes in *Salmonella typhi*, electrophoresis was carried out using agarose gel medium, after loading genes in to a 1.5% concentration agarose gel, the gel was submerged in a Tris/Borate/EDTA solution. Then the device was turned on after the cover was firmly put in place, with a voltage differential of 100 volts and a current of 80 amps, the transfer occurred for an hour.

Analysis of statistic

The chi-square test ($\chi 2$) was used to statistically analyze of the data based on [22].

Results

Isolation and diagnosis

This study examined the frequency of *Salmonella typhi* isolates that cause typhoid fever in patients based on the diagnosis of the specialist physician. In addition to examining virulence genes represented by the *fimH* and *csgA* genes, which are crucial components that enable bacteria to adhere to and invade host cells. Following the collection of 250 stool samples from typhoid fever patients, isolation and diagnosis results revealed that 174 (69.6%) of the total stool samples showed bacterial growth on the culture media used in the laboratory. While 76 (30.4%) samples had a negative culture result because no bacterial growth was visible on culture media, according to table (3), which shows the percentages of microbial culture on the culture media.

Table (3) shows the result of culturing stool samples on culture media

Bacterial growth on culture media	Number	Percentage
Positive culture cases	174	69,6%
Negative culture cases	76	30, 4%
Total	250	100%
X^2	76.83	
P value	<0.0001*	

* Highly significant difference at P<0.01

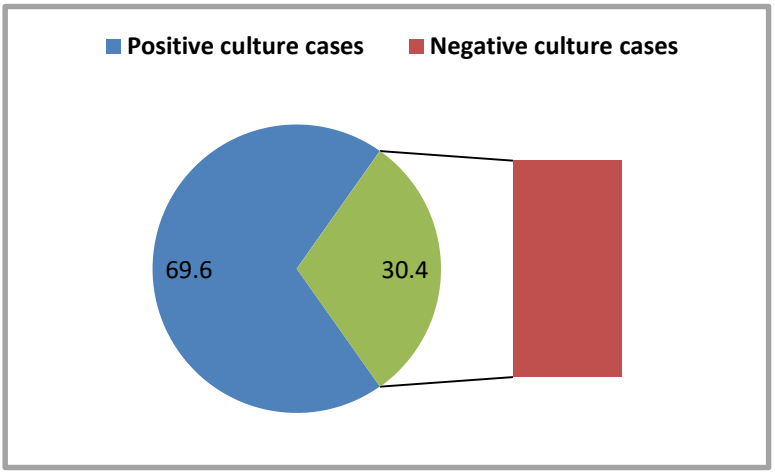

Figure (1) Distribution of culture-positive and culture-negative stool samples

The microbial diagnosis was carried out based on morphological features of the bacterial colonies on selective and differential culture media used in the study to investigation of *Salmonella* isolates. The bacterial isolation results were obtained were obtained after culturing stool samples on cultures media, on MacConkey agar medium, *Salmonella* colonies were colorless and did not ferment lactose. While *Salmonella* colonies appeared as colorless colonies with a black center on Salmonella-Shigella (SS) agar medium as a result of producing hydrogen sulfide H2S, They differ and distinguished from *Shigella* species because despite growing on Salmonella-Shigella (SS) agar medium, they do not produce hydrogen sulfide. And the culture results revealed that the

Salmonella colonies were red when grown on Xylose Lysine Deoxycholate (XLD) medium, and colony centers appeared black due to the production of H2S. After pure colonies were taken from bacterial cells isolated, treated with Gram stain and examined under a microscope, the results of microscopic analysis revealed that they appeared as rod-shaped cells that tested negative for Gram stain.

As shown in table (4), which displays the tests used to identify *Salmonella* isolates, the results of the biochemical tests show that the traits displayed by the isolated bacterial cells consistent with those of *Salmonella*. Following the addition of drops of hydrogen peroxide, colonies displayed bubbles, indicating that it was capable of producing catalase enzyme, in contrast, the colonies displayed a negative result for the oxidase test, as no color change transpired following the reagent's addition. As for the IMVIC test kit, the isolates gave a negative result for the indole because the addition of kovacs reagent did not produce a red ring at the top of the middle. The methyl red test yielded a positive result, indicating that the medium turned red as a result of the fermentation of sugar, additionally, voges-proskauer test produced a negative result. As for the growth test on simmon's citrate medium and use of citrates as the sole source of carbon and energy, colonies showed a positive growth result on this medium, as evidenced by the medium's color changing from green to blue. Also the colonies incapacity to change medium color from yellow to pink was demonstrated by the negative growth results of the colony culture on urea medium, this indicates that the medium's color has not changed due to an inability to produce urease enzyme. On the other hand, when grown on Triple Sugar Iron (TSI) medium, bottom of the medium turned yellow and surface appeared red, in the tubes holding the medium, the colonies also showed black coloring, which indicated the production of hydrogen sulfide. All isolates (99%) were identified as *Salmonella* by the VITEK test, a confirmatory test known for its accuracy. The suspected isolates were confirmed to be *Salmonella typhi* by serological testing with O and H antigens, which identify the antigens present in these isolates.

Table (4) Biochemical tests used to diagnose *Salmonella typhi*

Bacteria	Tests	Result
Salmonella typhi	Gram stain	-
	Catalase	+
	Oxidase	-
	Indol	-
	Methyl red	+
	Voges-Proskauer	-
	Simmon's Citrate	+
	Urease production	-
	H_2S Production	+
	Triple Sugar Iron (TSI)	+

(+): Positive (-): Negative

After completed of the diagnostic procedure, it was discovered that out of the 174 stool samples that tested positive for culture, 20 isolates of *Salmonella typhi* were isolated from the stool samples of patients who had typhoid fever. The percentage for this bacterium was 11.4%, while the remaining percentage was for other bacterial genera, the statistical analysis's findings revealed highly significant variations, as indicated in table (5) and figure (2).

Table (5) Percentage of *Salmonella typhi* isolated from stool samples

Organisms	Number	Percentage %
Salmonella typhi	20	11.4
Other Microorganisms	154	88. 5
Total	174	100
X^2	206.3	
P value	<0.0001*	

* Highly significant difference at P<0.01

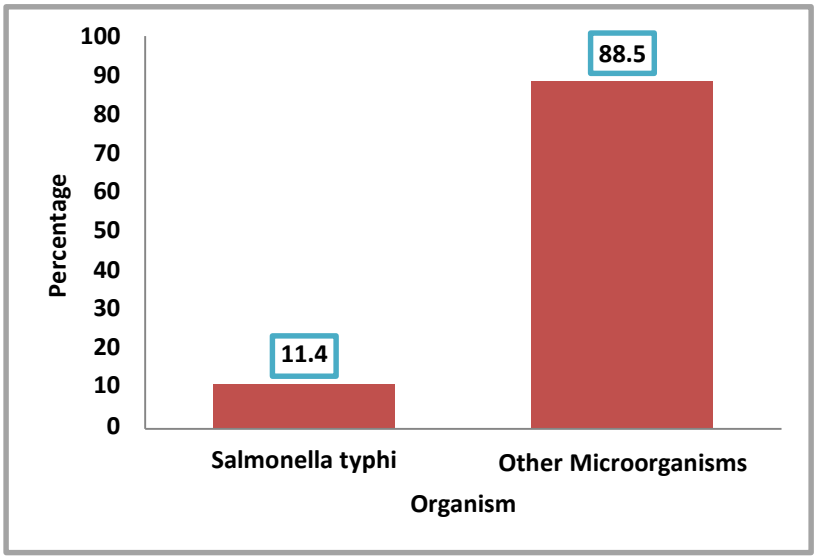

Figure (2) shows the prevalence of *Salmonella typhi* isolated from stool samples

Molecular characterization of virulence genes

The presence of *fimH* gene was investigated in the bacterial isolates, which is one of the genes that helps *Salmonella typhi* to adhere and enter host cells and cause infection. To detect the *fimH* gene, all *Salmonella typhi* isolates obtained from patient stool samples underwent polymerase chain reaction (PCR) testing. According to results from PCR analysis, UV light exposure, and *fimH* gene amplification, the *fimH* gene was found in the *Salmonella typhi* isolates at a rate of (100%), with a product size of (508 bp), it was discovered this gene was to be present in every *Salmonella typhi* isolates. This is shown in figure (3), which showing the *fimH* gene electrophoresis results. As for the *csgA* gene, by using PCR technology, the production of *csgA* gene was also examined in every isolate. *Salmonella typhi* isolates were found to contain this gene at a rate of 35% and with a product size of 418 bp based on the results of amplifying this gene after transferring it on a 1.5% agarose gel, a potential difference of 80 amps, and a voltage of 100 for an hour, it was found that only 7 isolates contained *csgA* gene, as shown in figure (4), which shows the result of *csgA* gene electrophoresis.

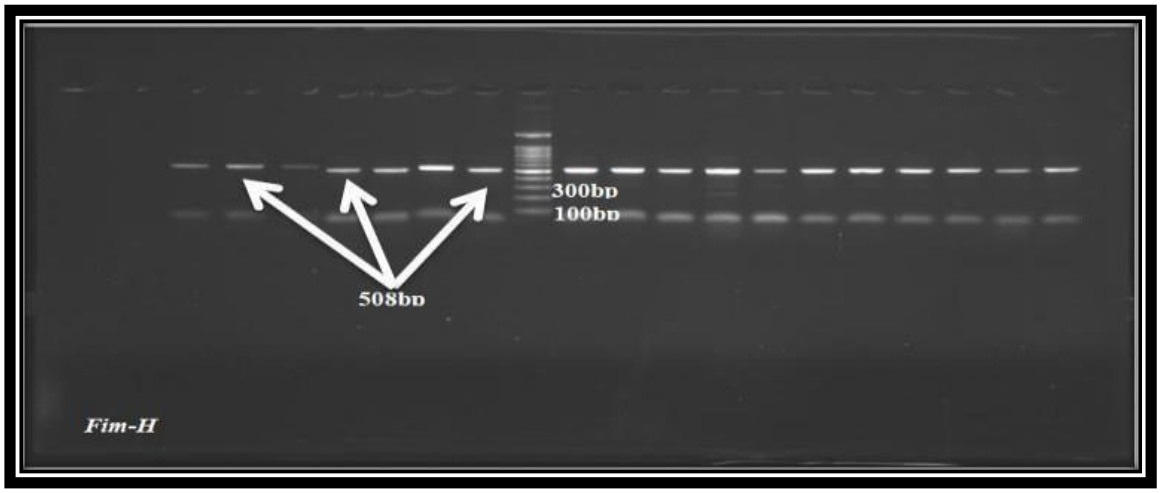

Figure (3): Agarose gel electrophoresis of *fimH* gene in *Salmonella typhi* isolates with product size 508bp

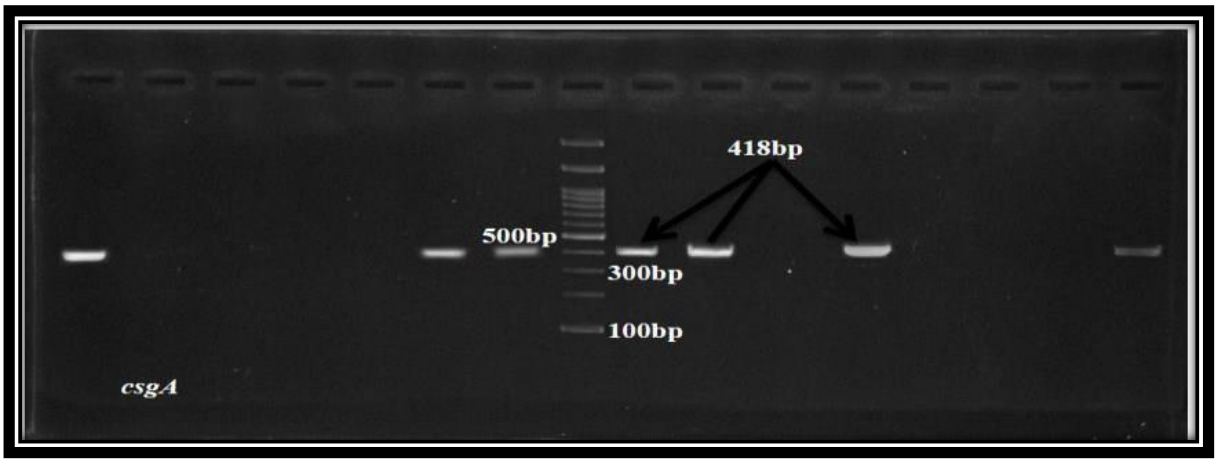

Figure (4): Agarose gel electrophoresis of *csgA* gene in *Salmonella typhi* isolates with product size 418bp

According to table (6) and figure (5), which display percentages of distribution of these genes, the statistical analysis revealed highly significant differences at the P<0.01 level. Indicating that the *fimH* gene is widely distributed in the isolates of *Salmonella typhi*, indicating its role in the adhesion process and assistance in invading the host, in contrast to the *csgA* gene, which appeared at a lower percentage in *Salmonella typhi* isolates.

Table (6) Distribution of virulence genes in *Salmonella typhi* isolates.

Genes	Number of positive isolations	Percentage (%)
fimH	20(20)	100
csgA	20(7)	35
X^2	24.54	
P value	<0.0001*	

* Highly significant difference at P<0.01

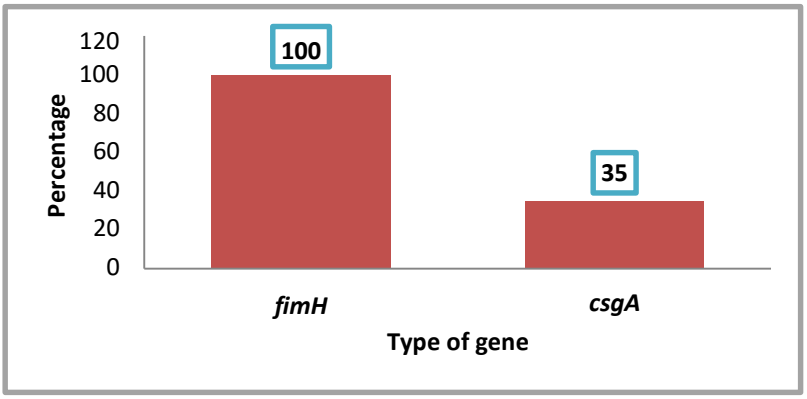

Figure (5) shows the prevalence of virulence genes in *Salmonella typhi*

Discussion

Salmonella is a pathogen that has a big influence on public health. It is an opportunistic bacterium that causes infection, and its spread is a threat to health [23]. Thus, the purpose of this study was to investigate its spread and to investigate the factors that contributes to its pathogenesis. According to the study's findings, 174 stool samples from typhoid fever patients had positive growth results (69.6%), whereas only 76 samples (30.4%) had no bacterial growth on the culture media for the samples initial culture. This rate of bacterial growth after culture of stool samples gives a clear proof that stool samples collected from the patients contain live bacteria. Regarding the stool samples that did not exhibit any microbial growth on the culture media used, this could be because the patients may had taken antibiotics prior to the test, which inhibits bacterial growth and prevents it from appearing on cultures media. Alternatively, it could be because other pathogens were present that the culture media used in this study were unable to detect. *Salmonella typhi* accounted for 11.4% of the total number of culture-positive stool samples which amounted to 174 samples, according to the diagnostic procedures of bacterial colonies that emerged on the differential and selective cultures media used to isolate *Salmonella typhi* and the ensuing biochemical and confirmatory tests. This isolation rate was in line with that of [24], who discovered that 12.0% of stool samples contained *Salmonella typhi*, [25] reported an isolation rate of 14.6% for this bacterium, which was comparable to our findings. Our findings were higher than those of [26], who discovered that *Salmonella typhi* was isolated from stool samples at a rate of 4.2%, also our findings were different from those of [27], who reported a higher isolation rate of *Salmonella typhi* reaching 72.5% than we obtained. When comparing our findings to those of other studies, the disparity in isolation rates could be attributed to variations in the number of samples examined, location of the study, ultimately, and the type of patients from whom the samples were obtained. In addition, some patients may taking antibiotics that have an effect on eliminating bacteria and thus affect the result of bacterial isolation. These results are consistent with [28] who indicated that the differences in prevalence of *Salmonella* depend on the type of patient, size of samples and geographic location.

PCR technique was used in molecular detection of the *fimH* and *csgA* genes in *Salmonella typhi* because it is an important molecular method for understanding the mechanisms that enable pathogens to cause infection. The results obtained when investigating virulence genes in *Salmonella typhi* isolates showed that the *fimH* gene was found in all isolates at a rate of 100%. The high frequency of *fimH* in *Salmonella typhi* indicates that the gene plays a vital role in the virulence of the bacteria in initializing infection, adhesion, and entry host cells and causing disease. This agrees with findings by [29], who identified *fimH* as an important factor in pathogenesis, with *fimH* aiding pathogens in their adherence to epithelial tissue. This is due to *fimH* being an adhesive protein, which enables pathogens to bind with their receptors [30]. These results were consistent with what was found by [31] who found that the *fimH* was present in 100% of *Salmonella* strains isolated from stool samples, while results of our study different from those of [32] in Dhi Qar Governorate, as he found that this gene was found only in 20% of *Salmonella*

isolates. As for *csgA* gene, this gene is responsible for the formation and production of curly fibers that play an fundamental role in adhesion and creation of biofilms, it was established that this gene is present in 35% of *salmonella typhi* because there was a variation in the possession of this gene by the isolates. Our results were different from those in [33], where they found that all *Salmonella typhi* isolates they studied had this gene. The absence of this gene in some isolates may be due to genetic mutations, which alter the gene expression of *csg* genes [34]. Our results show that some isolates of *Salmonella typhi* carried both genes, indicating that these isolates have capacity to both adhere and form biofilms, with the adhesion process being significantly influenced by *fimH* gene. As for *csgA* gene, it is responsible for the curly proteins that are essential for movement, colonization and survival, it also plays a part in the formation of biofilms by improving the surface-to-cell interactions that occur according to [35]. Therefore, compared to the isolates that do not contain these genes together, these isolates are more virulent and pathogenic, which allows the bacteria to survive, persist, resist environmental conditions and resist the effects of antibiotics. According to [36] *Salmonella* ability to cause severe infections and invade the host's tissues and cells is largely due to the virulence genes that possess. As a result, we discover that these genes are significant molecular virulence factors that contribute to *Salmonella typhi* pathogenicity, by identifying them can understand the mechanisms that facilitate infection and implement prevention measures.

Conclusion

We conclusion from the findings of this study, since *Salmonella typhi* was isolated from stool samples of typhoid fever patients, stool samples are a primary source for isolating *Salmonella typhi* and examining infections in the laboratory. According to the study, *fimH* gene essential for attachment and infection was present in all isolates, whereas 35% had the gene *csgA*, this implies that the isolates percentages of this gene differ. Additionally, it was discovered that some isolates have both genes, indicating that they are more persistent and alive. Therefore this study suggests conducting public awareness efforts in order to prevent infection through better hygiene behaviors, such as hand washing and food and beverage hygiene. Additionally, it recommends routine laboratory tests, including stool samples culture, to monitor the bacteria's progress and control the infection. Our research also recommends examining another virulence genes that these bacteria possess and correlating the presence of these genes to antibiotic resistance.

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Conflict of interests

No conflicts of interest

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Reference

1. J. Kaur, and S.K. Jain, “Role of antigens and virulence factors of *Salmonella enterica serovar typhi* in its pathogenesis”, *Microbiological Research*, vol.167, no. 4, pp.199–210, 2012. DOI: 10.1016/j.micres.2011.08.001.
2. P. N. Durga, P.D. Hari, and S. Jeongmin, “Enteric Fever Diagnosis: Current Challenges and Future Directions”, *Pathogens*, vol. 10, no. 4, pp. 410, 2021. DOI: 10.3390/pathogens10040410.

3. A. Akullian, E. Ng'Eno, A. I. Matheson, L. Cosmas, D. Macharia, B. Fields, and *et al*, "Environmental transmission of typhoid fever in an urban slum", *PLOS Negl Trop Dis*, vol. 9, no. 12, pp. e0004212, 2015. DOI: 10.1371/journal.pntd.0004212.

4. S. Brockett, M.K. Wolfe, A. Hamot, G.D. Appiah, E.D. Mintz, and D. Lantagne, "Associations among water, sanitation, and hygiene, and food exposures and typhoid fever in case-control studies: A systematic review and meta-analysis", *Am. J. Trop. Med. Hyg*, vol.103, no. 3, pp. 1020–1031, 2020. DOI: 10.4269/ajtmh.19-0479.

5. M. Khan, and S. Shamim, "Understanding the Mechanism of Antimicrobial Resistance and Pathogenesis of *Salmonella enterica Serovar Typhi"*, *Microorganisms*, vol. 10, no. 10, pp. 2006, 2022. DOI: 10.3390/microorganisms10102006.

6. R. Johnson, E. Mylona, and F. Gad, "Typhoidal Salmonella: Distinctive virulence factors and pathogenesis", *Cellular Microbiology*, vol. 20, no. 9, pp. 1-14, 2018. DOI: 10.1111/cmi.12939.

7. J. Chen, X. Zhou, Y. Tang, Z. Jiang, X. Kang, J. Wang, and M. Yue, "Characterization of two-component system CitB family in *Salmonella enterica* serovar Gallinarum biovar Gallinarum", *Veterinary Microbiology*, vol. 278, pp. 109659, 2023 DOI: 10.1016/j.vetmic.2023.109659.

8. L. Berrocal, and Et, A.l., "Stg Fimbrial Operon From *S. Typhi* STH2370 Contributes To Association And Cell Disruption Of Epithelial And Macrophage-Like Cells", *Biol. Res*, vol.48, no. 1, pp. 1-8, 2015. DOI: 10.1186/s40659-015-0024-9.

9. R. M. Kolenda, K. Ugorski, and Grzymajlo, "Everything you always wanted to know about *Salmonella* type 1 fimbriae, but were afraid to ask", *Frontiers in Microbiology*, vol.10, pp. 1017, 2019. Doi: 10.3389/fmicb.2019.01017.

10. K. H. Uchiya, Y. Kamimura, AJusakon, and T. Nikai, "Is Involved in Expression of Proinflammatory Cytokines in a Toll-Like Receptor 4-Dependent Manner", *Infect Immun*, vol. 87, no. 3, pp. e00881-18, 2019. Doi: 10.1128/IAI.00881-18.

11. C. Ou, M. Charles, and F. Dozois, "France Daigle Differential regulatory control of curli (*csg*) gene expression in *Salmonella enterica* serovar Typhi requires more than a functional CsgD regulator", vol.13, no. 1, pp. 14905. DOI: 10.1038/s41598-023-42027-y.

12. Y.J. Oh, M. B. Brenner, H. J. Gruber, Y. Cui, L. Traxler, C. Siliga, S. Park, and P. Hinterdorfer, Curli mediate bacterial adhesion to fibronectin via tensile multiple bonds. Scientific Reports, vol. 6, no. 33909, 2016. DOI: 10.1038/srep33909.

13. K. D. MacKenzie, M. B. Palmer, W. L. K¨oster, and A. P. White, "Examining the link between bioflm formation and the ability of pathogenic *Salmonella* strains to colonize multiple host species," *Frontiers in Veterinary Science*, vol. 4, p. 138, 2017. DOI: 10.3389/fvets.2017.00138.

14. D. L. Gibson, A. P. White, C. M. Rajotte, and W. W. Kay, "AgfC and AgfE facilitate extracellular thin aggregative fmbriae synthesis in Salmonella Enteritidis," Microbiology, vol. 153, no. 4, pp. 1131–1140, 2007.

15. J.F. MacFaddin, "Biochemical Tests for Identification of Medical Bacteria", 3th ed, Philadelphia: Lippincott Williams & Wilkins, 2000.

16. W. H. O. Salm-surv, and E.d. Global, June. "WHO Global Foodborne Infections Network From Food and Animal Faeces". 2010.

17. Brink, B. (2010). Urease test protocol. Washington, DC: American Society for Microbiology.

18. M. Cheesbrough, "*Salmonella* species. In District Laboratory Practice in Tropical Countries. Low-priced ed". Cambridge, pp.182-186, 2006.

19. M.J. Perilla, C. Bopp, J. Elliott, R. Facklam, T. Popovic, J. Wells, and World Health Organization. “Manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health importance in the developing world”, 2003.

20. J.R. Johnson, and A.L. Stell, “Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise”, *J Infect Dis,* vol. 181, no. 1, pp. 261–272, 2000. DOI: 10.1086/315217.

21. J. Schiebel, A. Böhm, J. Nitschke, and *et al*, “Genotypic and phenotypic characteristics associated with biofilm formation by human clinical Escherichia coli isolates of different pathotypes”, *Appl Environ Microbiol*, vol. 83, no.24, pp.:e01660-17, 2017. doi: 10.1128/AEM.01660-17.

22. J. Rahman, “Brief Guidelines for Methods and Statistics in Medical Research”. 1st edition. Springer Singapore Heidelberg, New York, Dordrecht, London, 2015.

23. B. Erdem, S. Ercis, G. Hascelik, D. Gur, A.D. Aysev, “ Antimicrobial resistance of Salmonella enterica group C strains isolated from humans in Turkey”, *Int J Antimicrob Agents*, vol. 26, no. 1, pp. 33–7, 2005. doi: 10.1016/j.ijantimicag.2005.03.007.

24. D. Ishaleku, and I. S. Kaigama, “Antibiotic Resistance of *Salmonella Typhi* from Stool of Patients Attending Selected General Hospitals in Nasarawa West Senatorial District Nasarawa State”, International Journal of Research and Scientific Innovvation, pp.2194-2203, 2025. DOI: https://doi.org/10.51244/IJRSI.2025.1215000168P.

25. A. R. Mohammed, “Antimicrobial Resistance Pattern of Salmonella Typhi from stool Culture”. *Chattagram Maa-o- Shishu Hospital Medical College Journal*, vol.14, no. 1, pp. 1-5, 2015. DOI: 10.3329/cmoshmcj.v14i1.22876.

26. T. Inusa, and S.M. Rabiu, A. Salawudeen, A.F. Umar, and E.B. Agbo, “Characterization of multidrug resistant Salmonella Typhi from clinical specimens”, *Gsc Biological and Pharmaceutical Sciences*, vol. 05, no. 02, pp. 053-058, 2018. DOI: 10.30574/gscbps.2018.5.2.0115.

27. M.E. Ohanu, “Typhoid fever among febrile Nigerian patients: Prevalence, diagnostic performance of the Widal test and antibiotic multi-drug resistance”. *Malawi Med. J*, vol.31, no. 3, pp. 184- 192, 2019. DOI: 10.4314/mmj.v31i3.4.

28. M. Tilahun, M. A. Belete, A. Gedefie, H. Debash, E. Alemayehu, D. G. Weldehana, H. Ebrahim, O. Mohammed, B. Eshetu, S. G.Tekele, Z.Mulatie, and A. Shibabaw. “Prevalence of *Salmonella* and *Shigella* species and their multidrug resistance patterns among pediatric populations in Ethiopia: a systematic review and meta-analysis”, BMC Infect Dis, vol. 25, no. 1, pp. 52, 2025. DOI: 10.1186/s12879-024-10425-w.

29. K.I. Uchiya, Y. Kamimura, A. Jusakon, and T. Nikai, “*Salmonella* Fimbrial Protein *FimH* Is Involved in Expression of Proinflammatory Cytokines in a Toll-Like Receptor 4-Dependent Manner”, *Amarican society for microbiology Journals /Infection Immunty*, vol. 87, no. 3, pp. e00881-18, 2019. Doi: 10.1128/IAI.00881-18.

30. M. Kuzminska-Bajor, K. Grzymajlo, and M. Ugorski, “Type 1 fimbriae are important factors limiting the dissemination and colonization of mice by *Salmonella enteritidis* and contribute to the induction of intestinal inflammation during Salmonella invasion”. *Front Microbiol*, vol.9, no. 6. pp. 276, 2015. Doi: 10.3389/fmicb.2015.00276.

31. H.F. Abbas, “Molecular Detection of Some Virulence Genes in *Salmonella* Species Isolated from Clinical Samples in Iraq”. *Archives of Razi Institute*, vol. 77, no. 5, pp. 1741-1747, 2022. DOI: 10.22092/ARI.2022.357983.2129.

32. A. Marza, B. Jasim, and K. Hanan, “Prevelance of Stnand FimHGenes in Salmonella enterica Isolated from Patients in Thi-Qar Governorate”. Vol. 24, no. 1, pp. 235- 242, 2025.

https://jige.uobaghdad.edu.iq/index.php/IJB/article/view/815 .

33. M. Riaz, S. Ahmad, F. Sattar, G. Li, Z. Din, S. Ahmad, A. Waheed, I. Ha, and T. Clark, "Whole-Genome Sequencing Analysis of Drug-Resistant *Salmonella Typhi* in Children", *Pathogens*, vol. 14, no. 10, pp. 1-14, 2025. 10.3390/pathogens14100967.
34. Z. Li , M. Zhang , G. Lei , X. Lu , X. Yang , and B. Kan , "A Single Base Change in the csgD Promoter Resulted in Enhanced Biofilm in Swine-Derived *Salmonella Typhimurium*", *Microorganism*s, vol. 12, no. 7, pp.1258, 2024. https://doi.org/10.3390/microorganisms12071258.
35. H. Steenackers, K. Hermans, J. Vanderleyden, and S.C.J. De Keersmaecker, "Salmonella biofilms: An overview on occurrence, structure, regulation and eradication". *Food Res. Int*, vol. 45, no. 2, pp. 502–531, 2012. https://doi.org/10.1016/j.foodres.2011.01.038.
36. S. Sotohy, and E. Khalifa, "Molecular Characterization of Some Virulence Genes of *Salmonella* enterica Serotype Sandow and Saintpaul Isolated from Environment of Dairy Farms at Assiut Province", Egypt. *J Vet Sci Technol*, vol. 09, no. 05, 2018. DOI: 10.4172/2157-7579.1000559.