Occurrences and Virulence Genes Detection of *Salmonella Typhi* Isolates Among Patients With Typhoid Fever

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Research Article

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Abstract

in low and middle-income countries, Typhoid fever is a major public health problem globally. The intensity of *Salmonella*'s pathogenesis is determined by the presence of numerous virulence factors expressed on *Salmonella* pathogenicity islands (SPIs). Study objectives included determining the occurrence of *Salmonella typhi* in typhoid fever by age, gender and determine the antibiogram profile against many routinely administered antibiotics. 166 samples of blood and feces were obtained from clinically suspicious patients at the Al-Najaf Public Health Department. This study took place between October 2020 and April 2021. Cultural and biochemical testing was used to identify the specimens, followed by identification using the Vitek2 system. The current work used a simple PCR technique, It was shown that genes are often associated with *Salmonella typhi* pathogenicity. Antibiotic sensitivity testing was performed on each isolate with the use 10 different antibiotics. A simple PCR experiment was performed to determine the presence of genes often associated with virulence in *Salmonella typhi*; 95(57%). This study identified two genes associated with pathogenicity (ctdB and tvIA). 95 (57%) of the 166 total isolates tested positive for ctdB, but additional virulence genes were discovered in all 71 (42.7%) tvIA (typhi Vi) gene isolated strains. This gene may contribute to the *S. typhi* strain's invasiveness. In conclusion, We demonstrated that typhoid infection is a significant public health risk, affects people of all ages, although it is more common in children aged 1-10 years. The rise of multidrug-resistant *Salmonella* spp. has created a significant issue for clinicians in terms of disease treatment. However, the current investigation discovered that the antibiotics Imipeneme and Cefotaxime are still effective against *Salmonella* spp. As a result, suitable criteria for prescribing these antibiotics should be followed to avoid multi-drug resistance concerns.

Introduction

Salmonella typhi and Paratyphi A, B, and C cause enteric fever (EF) that was previously widespread throughout Europe and North America. However, Due to improved sanitation and clean food and water, endemic EF in affluent countries declined precipitously. In many low-income countries, EF continues to be a severe concern, affecting both the endemic population and overseas travelers. Despite the general decreasing worldwide burden, pleomorphic clinical presentations, the development of *Salmonella* Paratyphi as a dominating pathogen in some places, the need for a more sensitive early diagnostic test, and widespread resistance to antibiotics pose a challenge. In most industrialized nations, imported travel-related infection is now the primary cause of EF [1]. Typhoid and paratyphoid fevers are examples of enteric fever. Serotypes A, B, and C of *Salmonella enterica* cause typhoid and paratyphoid diseases in humans. In low-income countries, its foodborne transmission, which is typically accompanied with inadequate hygiene and sanitation, promotes epidemics [2]. In poor countries, typhoid fever remains a serious health risk. Egypt, Pakistan, Syria and Iraq are high-risk locations for the spread of this disease [3]. *Salmonella enterica* serovar enterica, Gram-negative bacteria are responsible for typhoid fever. According to the MLST subtyping approach, the two most abundant *S. typhi* sequence types are ST1 and ST2 [4]. Typhoid fever has gradually diminished in popularity in industrialized countries because to its

rarity and extreme susceptibility to antibiotics such as third-generation cephalosporins, which have been considered the first line of infection therapy since the turn of the twentieth century [5].

The objective of this study was to evaluate the occurrence of *Salmonella typhi* in typhoid fever by age, gender and determine the antibiogram profile against many routinely administered antibiotics.

Material And Methods

2.1. Study Design

166 samples of blood and stool were acquired from Al-Najaf Health Directorate, Public Health Department who were clinically suspicious patients. This study was performed between October 2020 to April 2021.

2.2. Sample Collection

The blood and stool samples were collected from those patients sent to the laboratory in the morning session (9 AM to 12 PM) of patient examination. Venous blood sample (2–3 ml) was collected aseptically using 70% alcohol into a sterile test tube and centrifuged at 3000 revolutions per minute for 5 min to separate the serum. Fresh stool specimen provided by the febrile patient was put into a screw-capped container, labeled, and transported to Microbiology Laboratory of Al-Furat Al-Awsat Technical University, Kufa Technical Institute, Community Health Department for culture.

2.3. Widal agglutination test

Widal antigens (O) and flagellar antigens (H) were used to generate the Widal test for *Salmonella typhi* (U.K). Antibody titers in serum were detected using the Widal test. Through blood was collected, each patient had approximately 3 ml of blood collected and placed into sterile test containers. Centrifuge tubes at 3000 rpm for 5 minutes, A micropipette was used to collect the serum from each sample into a clean container. A sterile micropipette was used to transfer two drops of each serum sample to the microscope slide. The reagents for detecting *Salmonella* antigens (O) and (H) were also placed on the microscope slide. Applicator sticks were used to combine the two ingredients, and the tile was gently spun for one minute to ensure visible agglutination. Positive (+++)=1/320 antigens were detected between 0 and 15 seconds, (++)=1/160 antigens were detected between 15 and 45 seconds, and (+)=1/80 antigens were detected between 1 minute. while antigen not reacts were classed as negative (-). (more than one minute). Positive titers were defined as those greater than or equal to 1:80, whereas negative titers were defined as those less than 1:80 [6].

2.4. Samples Culture

2.4.1. Blood culture: Five milliliters of blood were aseptically placed into a sterile bottle containing fifty milliliters of sterilized Brain heart infusion (BHI) broth and incubated at 37°C for five minutes. The turbidity and color change of the blood culture were indicative of microbial growth constantly. Before

reporting a negative result, it is suggested that the culture for at least 7 days. After this, the bottle was thrown 14 days later (7). Subcultures were taken out as follows: a loopful of positive blood was transferred to MacConkey, Salmonella-Shigella agar (SS agar), and incubated at 37°C for 24 hours. Gram staining and light microscopy were used to examine the isolates [8]. Media were prepared according to the instructions of the manufacturers.

2.4.2. Stool culture: By the second week of infection, stool cultures are generally positive for typhoid fever. Most often, the stool is inoculated into fluid enrichment medium such as Tetrathionate or Selenite broth and then plated on Desoxy Cholate-Citrate Agar. It is possible to test for salmonella O antigens directly on culture plates using slide agglutination and sub-cultured to peptone water to determine the structure of the H antigen and do additional biochemical analysis [9].

2.5. Isolation and Identification of *Salmonella typhi*

Aseptically collected blood and stool samples in sterile BHIB containers. Subcultures on solidified sterilized XLD agar and MacConkey agar were created to assess growth after overnight incubation at 37°C. Colonies of *Salmonella* spp are often red with black center on XLD agar, but off white on MacConkey agar. In accordance with "Bergey's Manual of Determinative Bacteriology, 8th ed." taxonomic guidelines, the selected colonies were then identified as *Salmonella* spp. by microscopic and standard biochemical reactions such as TSI (Triple Sugar Iron), Urease test, MR-VP test, Oxidase test, Citrate utilization test, gas production [10].

2.6. Api20E system

Enterobacteriaceae and other gram-negative bacilli can be identified using this method. It is composed of twenty microtubes that hold a dehydrated medium (each microtube consists of a tube and cupul section). The Api20E system was installed and operated in accordance with the manufacturer's instructions.

2.7. Diagnosis by GN-ID with VITEK-2 Compact System

The test was performed in accordance with the manufacturer's specifications. This kit has recently been utilized for the fast detection of G+ve and G-ve bacteria.

2.8. DA extraction

The Genetic Material (DNA) of the *Salmonella typhi* strain investigated was purified using a kit provided by the Geneaid company in the United Kingdom.

2.9. PCR for the screening Virulence Genes of *Salmonella typhi*

PCR was used to examine for virulence genes in the *Salmonella typhi* isolates used in this investigation. There are two virulence genes that may be detected using primers, amplification products, and references in Table 1.

The detection procedure began with a 5-minute denaturation at 95 °C, followed by 30 cycles of denaturation at 95 °C for 45 s, annealing at 55 °C for 45 s, extension at 72 °C for 1 minute, and a final extension at 72 °C for 10 minutes. The *invA* gene fragment was amplified at a 63°C annealing temperature and extension for 1 minute. DNA ladders of 100 bp were used as molecular weight markers in the gel documentation system for electrophoresis 1.5 % agarose gels and ethidium bromide staining.

2.10. *Salmonella typhi* Susceptibility towards Antibiotic Tested

Salmonella typhi demonstration Susceptibility to various antibiotics was using disk diffusion test [13, 14]. To demonstrate the ability to cultivate pure colonies from previously identified bacteria, we added an isolated colony in culture to saline with the same density as (0.5) McFarland turbidity, which is essentially the same as the density of bacterial broth at 1.5×10^8 cells/ml. A sterile swab was used to inoculate the cells, which were then plated on Mueller - Hinton agar (MHA). Using burning forceps, antibiotic disks were put regularly over the medium surface and incubated for 18 hours at 37°C. The antibiotic inhibition zone was determined with the use of a specialized ruler. The diameter of the zone in comparison to the accepted clinical laboratory standards institute's standard results (CLSI).

2.11. Statistics Analysis

Data were analyzed using the SPSS (Statistical Package for Social Science) version 23 software, and significance was assessed at $P \leq 0.05$. using the Chi-squared test.

Result

As illustrated in Figure 1, 116 (69.9 percent) of the 166 patients were males and 50 (30.1%) were females (1).

Distribution of typhoid cases according to age and gender are shown in Table 2. The age group 1-10 had the highest rate of typhoid cases Additionally, males (44%) had a greater frequency than females (42%).

The most common months for *Salmonella typhi*-caused typhoid fever are August and September, with the lowest incidence occurring in January and February Figure 2.

This variability is expected considering that typhoid fever is a seasonal infection in Iraq, with the majority of cases occurring during the summer season. Iraq's average temperature ranges from 48°C. in July and August to below zero °C. in January.

As a result, people living in urban areas were more prevalent to typhoid fever than those living in rural areas, according to Table 3. This difference with other studies may be explained by rural lifestyles in which individuals drink unsterilized water making it easier for bacterial infections to spread through water.

The current work used a simple PCR technique to determine the presence of genes often associated with *Salmonella typhi* pathogenicity. At the molecular level, the *cdtB* gene was detected using a particular primer. As shown in Table 1, the *cdtB* gene was identified in 95 (57%) of *S. typhi* isolates with a long length (508 bp).

Antibiotic susceptibility testing revealed that *Salmonella typhi* samples increased their sensitivity to Imipeneme and Cefotaxime, Amoxicillin-clavulanic acid, and Chloramphenicol, but remained resistant to Ceftriaxone, Co-trimoxazole, and Ciprofloxacin. According to these findings, it can be concluded that Imipeneme and Cefotaxime are the more effective treatments for *Salmonella typhi*, Figure 3.

Discussion

Cultural, morphological, and biochemical tests were performed to identify microorganisms. These findings corroborate previous research [15], which found that males were more likely to be infected than females with diarrhea caused by these germs. I disagree with [22] that females were more likely to be infected with *Salmonella typhi* than males.

This could be due to their spontaneous actions, consumption of unsanitary food and water while outdoors, or a combination of the two [16]. These findings contradicted numerous research [17]. It has been discovered that the majority of infections occur between the ages of 21 and 30; Due to their increased immunity, adults (aged 40 and older) were shown to have a lower incidence of the disease [18].

This result is consistent with other studies performed in Iraq and elsewhere in the world. Typhoid fever was most prevalent in the summer months in Iraq and throughout the world and was less prevalent in other months of the year [19]. *Salmonella typhi* transmission through water is usually associated with a small inoculum and high attack rates over a short time, while the foodborne transmission is associated with a large inoculation and high attack rates over a short period. Summer's main risk factors for typhoid fever include eating homemade ice cream, increased water usage, and the domestic water supply is increasingly polluted with sewage, debris, and waste [20]. The high temperatures of summer may promote the spread of typhoid infection. The infection is transmitted by consuming contaminated food or water [21].

This toxin is different from other CDTs in that it is made of a combination of one *cdtB* and numerous PltB molecules, rather than the normal two CDT subunits (*cdtA* and *cdtC*) seen in *S. typhi*. While more studies of the underlying mechanism are required, it is thought that typhoid toxin contributes to the development of chronic *S.typhi* infection. Typhoid toxin has been implicated in the onset of symptoms and the transition from acute to chronic typhoid fever, according to these studies [22]. A drug targeting this toxin might help alleviate these symptoms. Cell death is caused by the toxin *cdtB*, which may be found in the cytolethal distending toxin gene (*cdtB*) [23]. The results of this research confirmed those of [24] who found the presence of the *cdtB* gene in all *Salmonella typhi* isolates Similarly, [25] discovered that the *cdtB* gene was present in all *Salmonella typhi* isolates. Of the 166 isolates tested, 42.7% tested positive for the *tvfA* (typhi Vi) gene. The *invA* gene of *Salmonella* comprises sequences unique to this species and

has been validated as a viable PCR target with the potential diagnostic accuracy [26]. This gene encodes a protein found in bacteria's inner membrane that aids in invasion of the host's epithelial cells [27].

This study contradicts the findings of [28] who discovered a 20% resistance rate to Ciprofloxacin. Ciprofloxacin was considered the antibiotic of choice following the introduction of MDR in *S.typhi* nonetheless, there have been numerous cases of Ciprofloxacin treatment failure in individuals with enteric fever. By previous CLSI breakpoints, these strains were Ciprofloxacin-susceptible [29]. Chloramphenicol sensitivity has been detected in 90% of *S. typhi* isolates from Haryana, according to MIC determinations [30]. Drug resistance to the causative agents of enteric fever is most likely caused by overprescribing and indiscriminate prescribing in the present study [31]. Additionally, it may be due to resistance genes being transferred (through plasmids) among intestinal bacteria or to chromosomal mutations strains resistant on the bacteria.. Recent decades have seen an increase in the prevalence of plasmid-encoded MDR, notably to the quinolones [32].

Conclusions

We demonstrated that typhoid fever is a significant public health risk, occurs in all age groups but is most prevalent in children aged 1-10 years. The emergence of multi-drug resistant Salmonella spp. is now a great challenge for physicians to treat the illness. But in the present study, it was found that the Imipeneme and Cefotaxime antibiotics are still working against Salmonella spp. So, appropriate guidelines should be followed to prescribe these antibiotics for avoiding multi-drug resistance problems.

Declarations

Funding:

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Conflicts of interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Availability of data and material:

Any additional information can be obtained from the corresponding author on request.

Code availability:

Not applicable

Authors' contributions:

Author Angham Najah Al-Khafaji contributed to the writing of the research.

Ethics approval:

The study was approved by the Research Ethics Committee of the Kufa Technical Institute, Al-Furat Al-Awsat Technical University.

Consent to participate:

Not applicable.

Consent for publication:

Not applicable.

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Tables

Table 1. PCR primers used for amplification of virulence genes in Salmonella typhi

Virulence Genes		Primer sequence(5'-3')	Size bp	Reference
<i>cdtB</i>	F	TAAGTGGTACTGCCGGTGTG	508	[11]
<i>cdtB</i>	R	GTAGGTGCGAGTACGGCTAC		
<i>invA-1</i>	F	TTGTTACGGCTATTTTGACCA	521	[12]
<i>invA-2</i>	R	CTGACTGCTACCTTGCTGATG		

Table 2. Age and gender distribution of typhoid cases

Age group	Male		Female	
	Number	Percentages %	Number	Percentages %
1-10	51	44	21	42
11-20	30	25.9	16	32
21-30	21	18.1	5	10
31-40	7	6	5	10
41-50	5	4.3	2	4
51-60	2	1.7	1	2
Total	116	100	50	100

Table 3. Prevalence of *Salmonella typhi* isolation according to residence

Residence	No. of Specimen examined	Percentage %
Urban	97	58%
Rural	69	42%
Total	166	100%

Figures

Figure 1

Distribution of patients according to gender

Figure 2

Figure 3

Antibiotic susceptibility pattern of *Salmonella typhi*