Journal of Applied and Natural Science
16(4), 1476 - 1484 (2024)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Molecular identification of *CdtB* and *TviA* virulence genes of *Salmonella typhi* isolated from cholelithiasis patients in Erbil City, Iraq

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Article Info
https://doi.org/10.31018/jans.v16i4.5922
Received: July 10, 2024
Revised: November 4, 2024
Accepted: November 10, 2024

How to Cite
Mustafa, A. H. and Raad, A. Z. (2024). Molecular identification of *CdtB* and *TviA* virulence genes of *Salmonella typhi* isolated from cholelithiasis patients in Erbil City, Iraq. *Journal of Applied and Natural Science*, 16(4), 1476 - 1484. https://doi.org/10.31018/jans.v16i4.5922

Abstract
Gallstone disease (GSD), particularly cholecystitis accompanied by gallstones, is a prevalent global health concern with significant morbidity and mortality rates. Typhoid fever is a public health issue in low- and middle-income countries. The severity of the pathogenesis depends on *Salmonella's* possession of several virulence factors encoded on Salmonella pathogenicity islands (*SPIs*). This study aimed to isolate and identify *Salmonella typhi*, the causative agent of typhoid fever, in clinical samples from cholelithiasis patients in Erbil City, Iraq. A cross-sectional study was conducted from October 2023 to March 2024, involving 125 patients diagnosed with gallbladder disease. Gallstone, bile, and gallbladder tissue samples were collected and analyzed for bacterial growth. Biochemical and cultural studies confirmed antibiotic susceptibility and Vitek2 system identification. The present study used a traditional PCR assay to detect *Salmonella typhi* pathogenicity genes. This study found the virulence genes *CtdB* and *TviA* using specific primers. Out of 125 samples, 101 (80.8%) showed bacterial growth, with 8 (6.4%) positive for *S. typhi* and 93 (74.4%) positive for other bacterial species. Gallstones had the highest proportion of *S. typhi* isolation 5 (4%). Antibiotic susceptibility testing revealed significant resistance to commonly used antibiotics like ampicillin, ciprofloxacin, chloramphenicol, and trimethoprim-sulfamethoxazole. However, the strains demonstrated better susceptibility to various antibiotics. The molecular result showed the virulence genes, including *CtdB* and *TviA,* were detected in all (100%) isolated strains. *S. typhi's* virulence characteristics significantly contributed to gallbladder infections and multidrug-resistant strains (MDR).

Keywords: *Salmonella typhi*, Cholelithiasis, Polymerase chain reaction (PCR), Virulence genes

INTRODUCTION

Salmonella, a substantial genus with significant global health implications, is the primary culprit behind foodborne illnesses, resulting in numerous fatalities worldwide (Lee *et al*., 2015). This formidable pathogen poses a severe threat to individuals across the globe, leading to substantial morbidity, mortality, and economic repercussions (Atlaw *et al*.,2022; Uzairue and Shittu,2023).

Salmonella enterica consists of more than 2668 serovars. It can cause humans and animal disease (Saleh *et al*.,2016). This pathogen enters through contaminated food and drink. Once within the body, it might enter the small intestine or circulation. From there, it can reach the liver, gallbladder, spleen, lungs, and other organs (Muralidhar,2019).

Typhoid fever, a dangerous illness, is caused by *Salmonella typhi*. It is one of the biggest problems in the world, especially in developing nations where the disease is endemic, and Iraq is one of the countries that experience this disease on an annual basis (EL Morabet *et al*.,2023). Humans are the only host of this bacteria (Chia *et al*.,2009). When someone consumes food or liquids tainted with bacteria, they become infected. It can also spread from person to person. It claims the lives of approximately 128,000 to 161,000 individuals worldwide each year, with an estimated 11 to 20 million people contracting the infection annually. The endemic nature of typhoid fever can be attributed to persistent hot weather conditions along with frequent disruptions in electricity and water supply (Sadeq and Rasha,2017; EL Morabet *et al*.,2023).

Systemic *Salmonella* infections include several protective and offensive virulence factors due to their complex pathophysiology. All of these assist the human intracellular pathogen's invasion, replication, and sur-

vival (Hanan *et al*.,2021). This microorganism can breach the intestinal epithelial barrier, infiltrate macrophages, and disseminate throughout the body. Upon establishing colonization in the liver, *S. typhi* can be excreted into the gallbladder, leading to chronic colonization of the gallbladder wall. Approximately 3-5% of individuals infected with *S. typhi* develop chronic carrier status, with the gallbladder serving as the primary site of long-term persistence. Notably, persistent *S. typhi* colonization is linked to the developing gallstones and gallbladder cancer (AL-Sanjary and AL-Rawi,2022; Gonzalez *et al*.,2022; Rehman *et al*.,2023).

Biofilm formation usually enhances gallbladder colonization and *S. typhi* growth on gallstones. Research shows that bile and cholesterol increase bacterial adhesion in gallstones (Gonzalez-Escobedo and Gunn,2013).

Salmonella has many virulence techniques to bypass host defenses. *Salmonella* pathogenicity islands (*SPIs*) contain the virulence and intracellular survival genes essential for *Salmonella* invasion and survival. Large gene cassettes called *SPIs* translate host-specific factors, including bacterial virulence factors (Sotomayor Castillo,2017). These include cytolethal distending toxins (CDT), flagella, fimbriae, and pathogenicity islands, representing notable genetic components within the bacterial chromosome. The *Salmonella* chromosome contains a sizable genome that includes the virulence genes necessary for the pathogen to invade, reproduce, and withstand host defenses to remain inside the host cell (Di Domenico *et al*.,2017; Rajab and Turki,2021).

Salmonella typhi utilizes cytolethal-distending toxins (CDTs) as significant virulence factors. The *CdtB* gene, responsible for the cytolethal distending toxin, encodes toxins that prompt apoptosis in infected cells. SPI-11 encodes the *S. typhi* serotype-specific *CtdB* gene. *S. typhi* produces most of its toxin in the *Salmonella*-containing vacuole inside the cell and releases it (Johnson *et al*.,2018; Hassena *et al*.,2021). The Vi antigen, flagella, pilli, and other host invasion genes depend on the *TviA* gene. The isolate with the *TviA* gene may include the pathogenic island SPI-7, specific to *S. typhi*. Sometimes the isolate may form biofilm and is Vi-positive (Zhang *et al*.,2018; Kerim *et al*.,2023).

This investigation aimed to detect and isolate *Salmonella typhi*, the typhoid fever-causing bacterium, using clinical samples obtained from patients suffering from cholelithiasis in Erbil City, Iraq.

MATERIALS AND METHODS

Study design

The Identification of *Salmonella typhi* genes for cholelithiasis patients in Erbil City was done based on an observational study. The cross-sectional part of the study was conducted between October 10, 2023 and March 10, 2024.

Participants

The participants were 125 patients, aged between 15 and 80 years with gallbladder disease treated by open or laparoscopic cholecystectomy at Erbil Teaching Hospital and Rizgari Teaching Hospital in Erbil City. These individuals had a history of upper quadrant and epigastric pain within the past three months. They had been diagnosed with asymptomatic gallstones using abdominal ultrasound as the standard diagnostic tool.

Screening and characterization of *S. typhi* persisting in gallbladder

Sample collection

All fresh specimens (gallstones, bile, and gallbladder tissue samples) were collected from patients undergoing cholecystectomy and transferred to special, sterile containers. Immediately after collection, samples were sent to the laboratory for further processing.

Bacterial cultures

Gallbladder samples were incubated at 37°C for one day in sterile vials with 5 mL of tryptone soya broth. After one night in enrichment broth, it was carefully transfered to Petri dishes plates with blood, MacConkey, *Salmonella-Shigella* (S-S), and Xylose Lysine Deoxycholate (XLD) agars. Incubating the plates at 37 °C for a period of 24-48 hours. After the incubation period, the colonies' morphological and microscopic characteristics were examined. The colonies on the Petri dishes plates underwent multiple subcultures on selective solid media to obtain pure cultures of the microorganisms. The morphological characteristics of the microorganisms were observed on selective media to identify the microorganisms in pure culture. Microscopical inspection stained with Gram-stain allowed for the exact form and kind of reaction to be observed throughout the diagnostic process after acquiring of bacterial isolates.

Biochemical tests

The crucial biochemical tests were carried out, which comprised the following assays: triple sugar iron (TSI), catalase, oxidase, urease, and citrate utilization.

VITEK-2 compact system

To diagnose the bacteria isolated from various types of gallbladder samples, the automated VITEK-2 compact system (BioMerieux, Paris, France) is used, following the manufacturer's instructions, to test *Salmonella typhi* that has been identified using morphological and biochemical testing.

Antibiotic sensitivity test

Antibiogram analysis was carried out through the Kirby-

Bauer disc diffusion protocol as discussed in the Clinical and Laboratory Standards Institute (CLSI, 2023). Bacterial dilution was prepared and compared with Mac Farland 0.5 standard. Dilution was poured and spread over on Mueller Hinton agar media. Antibiotic discs were placed over the media with help of forceps. It was kept at 37ºC for 24 hours in an incubator. A scale was used to measure the zone of inhibition.

Molecular study

The presence of virulence genes, including *CdtB* and *TviA*, in the *Salmonella typhi* isolates utilized in this study was investigated using the traditional polymerase chain reaction (PCR) technique. Specifically, two virulence genes were targeted for detection using specific primers and amplification process conditions.

Extraction of genomic DNA

The American company Geneaid's genomic extraction tool extracted the bacteria's DNA. Company guidelines were followed during extraction. The extracted nucleic acid was tested for purity using a nanodrop spectrophotometer, which measures concentration (ng/µL) by measuring absorbance at 260-280 nm.

Agarose gel electrophoresis of extracted DNA

The 1.5g of agarose gel was dissolved in 100 ml of sodium borate (SB) buffer at 1× concentration on a heat plate for 15 minutes to dissolve. Following cooling at 50°C, 3 µL of Red Safe stain was applied and stirred. The gel was placed in the electrophoresis tray and solidified at room temperature for 15 minutes. Then, the comb was gently removed from the gel to leave wells for injecting samples.

Primer pairs preparation

The primers and the DNA sequence initiator were designed using the genetic sequence in the GenBank according to the sources mentioned above on the National Biological Information website (NCBI) using the primer design program (Table 1). These primers were prepared by the Canadian company (IDT) and were used according to the manufacturing instructions by adding deionized distilled water (dd H_2O) to the dried tube containing the primers according to the size fixed on the tube. Then, they were mixed well with a vortex device to obtain a solution. Finally, they were stored until use at a temperature of -20 °C.

Preparation of the polymerase chain reaction (PCR) master mix

The PCR mixture was prepared utilizing the Intron kit from Korea, along with the supplementation of the AccuPower PCR PreMix kit. The PCR reaction mixture was created by combining the extracted DNA, primers, dNTPs (deoxynucleotide triphosphates), Taq polymerase, $MgCl_2$ buffer, and deionized distilled water (ddH_2O). The tubes were sealed, and vortex mixed for

Table 1. Virulence genes primers sequences with their amplicon size base pair (bp)

Genes	Primer sequence (5'- 3')	Size bp	Reference
CdtB F	TAAGTGGTACTGCCGGTGTG	508	(Hasson and Abady,2019)
CdtB R	GTAGGTGCGAGTACGGCTAC		
TviA F	GTTATTTCAGCATAAGGAG	599	(Liaquat *et al*.,2018)
TviA R	ACTTGTCCGTGTTTTACTC		

Table 2. PCR reaction conditions to detect the genes used in this study

TviA gene				
Steps	**Temp. °C**	**Time**	**Cycle**	**Reference**
Initial denaturation	95	5 min	1	(Albanwawy and Abdul-Lateef,2021)
Denaturation	95	30 sec	30	
Annealing	50	30 sec		
Extension	72	1 min		
Final extension	72	5 min	1	
CdtB gene				
Steps	**Temp. °C**	**Time**	**Cycle**	**Reference**
Initial denaturation	95	5 min	1	(Albanwawy and Abdul-Lateef,2021)
Denaturation	95	30 sec	30	
Annealing	55	30 sec		
Extension	72	1 min		
Final extension	72	5 min	1	

10 seconds after the mixture was created. The tubes were then placed in a PCR thermocycler to amplify target genes.

Polymerase chain reaction (PCR) assay

To confirm *S. typhi*, thermal cycle PCR was used. This method requires *CdtB* and *TviA* gene primers and sequencing information. The reaction tubes were transferred to the thermo cycler apparatus according to the programs shown in Table 2.

Statistical analysis

IBM SPSS ver. 23 was used for our statistical study. The chi-square test was used to determine the statistical differences among the various groups, and a probability of $P < 0.05$ was deemed to indicate statistical significance.

Ethical approval

The study was conducted using ethical approval obtained from the Scientific Research Ethics Committee at the College of Medicine, Tikrit University, No. 3/7/194 on 4/10/2023. Informed consent was obtained from all participants involved in the study. Confidentiality of participant information was ensured, and data were anonymized during analysis and reporting.

RESULTS

Table 3 presents the distribution of bacterial growth in different types of gallbladder samples (gallstones, bile, and gallbladder tissue) obtained from 125 patients. Of 125 samples, 101 (80.8%) showed bacterial growth, while 24 (19.2%) had no bacterial growth. Among the 101 samples with bacterial growth, 8 (6.4%) were positive for *S. typhi*, and 93 (74.4%) showed growth of other bacterial species. Gallstones had the highest proportion of samples, with bacterial growth 57 (45.6%), followed by bile 31 (24.8%) and gallbladder tissue 13 (10.4%). *S. typhi* was the predominant organism isolated from gallstones 5 (4%), bile 1 (0.8%), and gallbladder tissue 2 (1.6%). The chi-squared test result (p-value = 0.590) indicates no significant difference in the distribution of bacterial growth among the three sample types.

Table 4 shows that out of 75 gallstone samples, 5 (4%) were culture a positive for *S. typhi*. For bile samples, 1 out of 35 (0.8%) was positive. Regarding gallbladder tissue, 2 out of 15 samples (1.6%) were positive for *S. typhi*. In total, 8 out of 125 samples (6.4%) were culture -positive for the bacteria. The chi-squared test revealed a significant difference ($p = 0.043$) between the groups, indicating a potential association between the sample source and the presence of *S. typhi*.

The antibiotic sensitivity test results shown in Table 5 demonstrate the varying degrees of susceptibility and resistance of *Salmonella typhi* strains isolated from cholelithiasis patients against 14 antibiotics. The data reveals a concerning trend of high resistance rates, with 75% of the strains exhibiting resistance to ampicillin, ciprofloxacin, and chloramphenicol. Also, 62.5% of the strains were resistant to trimethoprim-sulfamethoxazole. Notably, 50% of the strains were resistant to ceftriaxone and ceftazidime. However, the strains showed relatively better susceptibility to amikacin, azithromycin, tetracycline, levofloxacin, and ofloxacin, with 75% susceptible to these antibiotics. The chi-squared test yielded a significant p-value of 0.0115, indicating a statistically significant difference in the distribution of susceptibility patterns among the antibiotics tested.

Figure 1 shows the results of a polymerase chain reaction (PCR) amplification of the *CdtB* gene from *Salmonella typhi*. The image displays eight lanes labelled 1 through 8, each representing a sample containing the amplified *CdtB* gene. A single bright band in each of these lanes indicates successful PCR amplification, with the amplicon (PCR product) having the expected size of 508 base pairs (bp), as indicated by the white lines. Lane M represents a 100 bp DNA ladder, a molecular weight marker used to estimate the size of the DNA fragments in the other lanes.

According to the Fig. 2 caption, the expected size of the *TviA* gene amplicon is 599 base pairs (bp). By comparing the position of the white lines in lanes 1-8 with the DNA ladder in lane M, the results revealed that all 8 *S. typhi* isolates (100%) tested positive for this gene. Notably, the amplified product exhibited a consistent length of 599 base pairs. It can be confirmed that the PCR amplification was successful and that the amplified product has the expected size of 599 bp .

DISCUSSION

The present study showed that bacterial growth varied across sample types, with gallstones exhibiting the highest proportion (45.6%) followed by bile (24.8%) and gallbladder tissue (10.4%). The chi-squared test revealed no statistically significant difference. The non-significant p-value of 0.590 indicates no substantial evidence of an association between bacterial type distribution and sample source or growth conditions. This result disagrees with Hawar *et al*. (2019), which showed bacterial isolates in different gallbladder samples (mucosa 42.35%, gallbladder sac 22.35 %, gallstones 20%, and bile 15.29%). The study demonstrated the isolation of *S. typhi* from 5, 2, and 1 samples of gallstones, gall bladder epithelial tissues, and bile, respectively. These findings align with a previous study by Mansour *et al*. (2012), which reported upbeat results for *S. typhi* in approximately 28, 12, and 4 samples of gallstones, gall bladder epithelial tissues, and bile, respectively.

Table 3. Distribution isolated sample according to sources and growth of bacteria

Type of gallbladder sample	Gallbladder sample		Bacterial growth		Other types of bacterial growth		*S. typhi* growth		No bacterial growth	
	No.	%	No.	%	No.	%	No.	%	No.	%
Gallstone	75	60	57	45.6	52	41.6	5	4	18	14.4
Bile	35	28	31	24.8	30	24	1	0.8	4	3.2
Gallbladder tissue	15	12	13	10.4	11	8.8	2	1.6	2	1.6
Total	**125**	**100**	**101**	**80.8**	**93**	**74.4**	**8**	**6.4**	**24**	**19.2**

Chi-squared test (2.809) p-value (0.590) NS: Nonsignificant difference between groups.

Table 4. Distribution of *Salmonella typhi* bacteria samples according to sources' numbers and percentages

Type of gallbladder sample	Number of samples	Culture positive for *S. typhi*		Chi-squared test (p-value)
		No.	%	
Gallstone	75	5	4	1.66 (0.043 S)
Bile	35	1	0.8	
Gallbladder tissue	15	2	1.6	
Total	**125**	**8**	**6.4**	

S: Significant difference between groups (p-value < 0.05).

Table 5. Antibiotic sensitivity test (Disc diffusion test) for *Salmonella typhi* isolated from cholelithiasis patients (Number of *S. typhi* = 8)

No.	Antibiotics	Number of susceptible (S) strains No. (%)	Number of intermediate resistant (I) strains No. (%)	Number of resistant (R) strains No. (%)
1	Amikacin	6 (75%)	1(12.5%)	1(12.5%)
2	Ampicillin	2(25%)	0	6 (75%)
3	Amoxycillin / clavulanic acid	4(50%)	2(25%)	2(25%)
4	Azithromycin	6 (75%)	2(25%)	0
5	Cefotaxime	2(25%)	3(37.5%)	3(37.5%)
6	Ceftriaxone	3(37.5%)	1(12.5%)	4(50%)
7	Ceftazidime	4(50%)	0	4(50%)
8	Ciprofloxacin	1(12.5%)	1(12.5%)	6(75%)
9	Tetracycline	6(75%)	0	2 (25%)
10	Levofloxacin	6 (75%)	1(12.5%)	1(12.5%)
11	Ofloxacin	6 (75%)	1(12.5%)	1(12.5%)
12	Trimethoprim-sulfamethoxazole	3(37.5%)	0	5(62.5%)
13	Chloramphenicol	2(25%)	0	6 (75%)
14	Gentamycin	5(62.5%)	1(12.5%)	2(25%)
Chi-squared test (p-value)		45079. (0.0115 S)		

S: Significant difference between groups (p-value < 0.05).

The present study showed that from a total of 125 samples of the gallbladder from GD patients, only 8 (6.4%) patients were positive for *S. typhi*, about 93 (74.4%) patients were positive for other types of bacterial infections, and 24 (19.2%) patients had no bacterial growth. The study findings reveal a statistically significant difference in the prevalence of *Salmonella typhi* across various gallbladder sample types. Gallstones were most *S. typhi*-positive, followed by gallbladder tissue and bile. Results imply a link between the sample source and *S. typhi* detection. Gallstones are more likely to harbor typhoid fever's cause. These findings emphasize the relevance of sample type for testing gallbladder specimens for *S. typhi*. The present study's findings on *S. typhi* prevalence in cholelithiasis patients in Erbil City, Iraq, are consistent with those of several Iraqi studies, including Sadeq Noomi and Ayad Majeed (2018), which found 6%, and Shareef (2005), which found 6.6%. The present results contrast from others showing higher or lower *S. typhi* isolation rates (Capoor *et al*.,2008; AL-Zuharri,2011; Thapa *et al*.,2016). Isolation rates vary due on location, sample size, and laboratory methods.

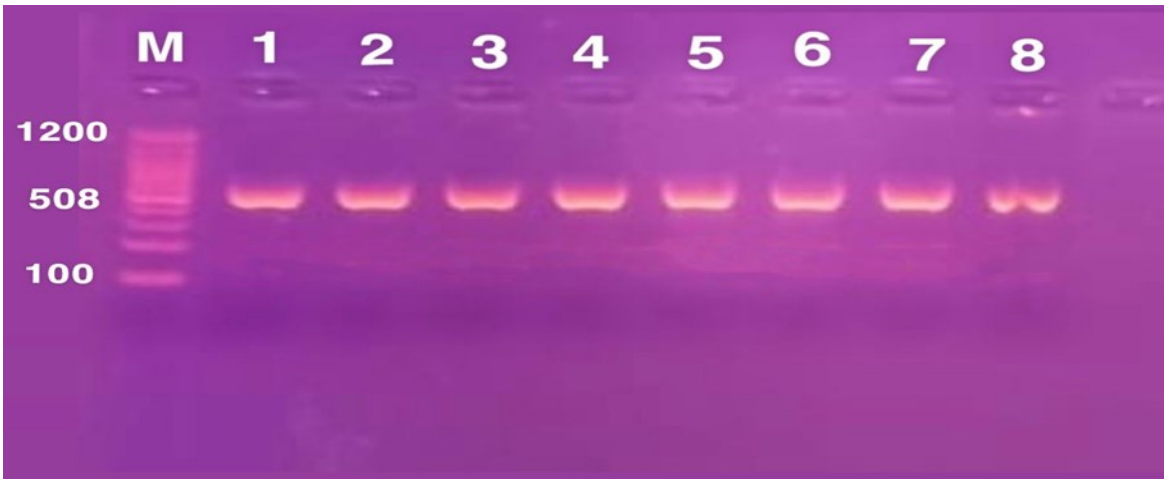

Fig. 1. *Agarose gel electrophoresis of PCR product obtained with Salmonella typhi using CdtB-specific primers. Lanes 1-8 represent the identified CdtB gene; Lane M represents the 100bp DNA ladder. The size of the product is 508 bp for the CdtB gene*

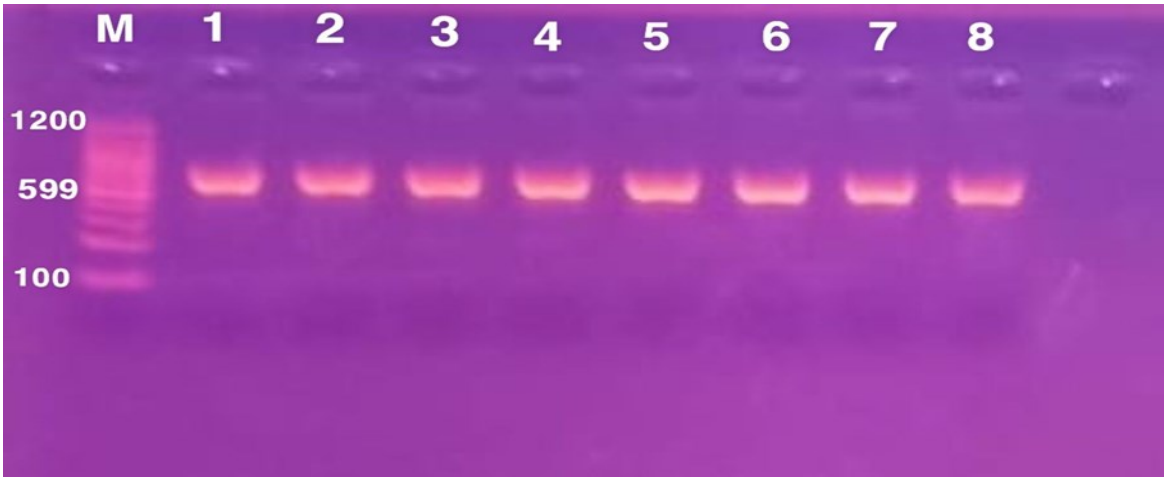

Fig. 2. *Agarose gel electrophoresis of PCR product obtained with Salmonella typhi using TviA-specific primers. Lanes 1-8 represent the identified TviA gene; Lane M represents the 100bp DNA ladder. The size of the product is 599 bp for the TviA gene.*

Our data range within the reported 1% to 34% incidence of *S. typhi* isolates from gallstone patients, indicating gallbladder disease as a *Salmonella* risk factor (Dolecek *et al*.,2008). These findings emphasize the need to monitor and characterize *S. typhi* isolates to enhance public health and patient outcomes.

Salmonella typhi is linked to gallstones due to its ability to produce beta-glucuronidase in bile salts, which breaks down bilirubin diglucuronide in bile. This leads to the formation of brown pigment stones (Cetta,1986), which are formed by combining bilirubin with calcium under acidic conditions. The bacteria can also secrete phospholipase enzymes, which break down phospholipids into free palmitic acid and calcium palmitate. They stick to the solid pigment of calcium bilirubinate through having capsule glycocalyx (Stewart *et al*.,1987). The present study shows that *Salmonella* can create biofilms on human gallstones and bile increases this process. The asymptomatic carriers for instance, can potentially live without clinical manifestations, and that renders traditional antibiotics ineffective. Gallstones can cause mechanical inflammation, which results in chronic inflammation. *Salmonella typhi's* relationship with gallstones is that form biofilms and alter gene expression (Koshiol *et al*.,2016).

Antibiotic susceptibility patterns of *S. typhi* isolated from patients

Antibiotic susceptibility test results indicated the *S. typhi* isolates had multiple drug resistance profiles and exhibited variation in antibiotic sensitivity. Resistance profiling was notably different between the two groups based on the pre-test calculated chi-squared statistical results; regular resistance surveillance and prudent use of antibiotics are recommended to combat persistent antimicrobial resistance and guarantee successful treatment outcomes. In concordance with the study done by AL-Turaihi *et al*. (2023); Sahu *et al*. (2021), it was revealed that *S. typhi* isolates could survive in ampicillin and chloramphenicol. *S. typhi* is known to be

resistant to ciprofloxacin and trimethoprim-sulfamethoxazole as evidenced by the studies before this one, which were done by Hanan *et al*. (2021); Radi *et al*. (2023). In this study, all *S. typhi* isolates were sensitive to amikacin, azithromycin, levofloxacin, and tetracycline. These findings confirm the study conducted by Al-Aarajy (2022).

Due to improper administration or sale without prescription in retail markets and private pharmacies, many antibiotics have a high resistance incidence among bacterial isolates (Gilmore and Denyer,2023). Violence, abuse, antimicrobial effectiveness, control, and bacterial virulence factors contribute to resistance. Vertical transfer of antimicrobial resistance genes and conjugative transfer of bacterial plasmids also contribute. Drug-resistant *Salmonella typhi* strains can also result from incomplete antibiotic therapy (Parry *et al*.,2015).

Molecular detection of *Salmonella typhi* virulence genes

These findings imply that *S. typhi* strains from the study population carry the *CdtB* gene, possibly contributing to their pathogenicity and severe infections. This finding confirmed a prior study conducted by Albanwawy and Abdul-Lateef (2021), showing that *Salmonella typhi* isolates had the *CtdB* gene. It also aligns with another study by Thakur *et al*. (2019), indicating that all *S. typhi* isolates have the *CtdB* gene.

Salmonella Pathogenicity Island-11 genes generate typhoid toxin, a unique *S. typhi* toxin. Only *Salmonella*-containing vacuole (SCV) macrophages express it (Johnson *et al*.,2018). The *CdtB* gene produces a toxin that kills infected cells (Gonzalez-Escobedo and Gunn,2013). A single *CdtB* molecule, one *PltA* molecule, and numerous *PltB* molecules make up the typhoid toxin, unlike other CDTs. However, it lacks *CdtA* and *CdtC* (Albanwawy and Abdul-Lateef,2021). Various mammalian cell types die from CDT activity via apoptosis. Typhoid toxin may affect *S. typhi* infection progression from acute to chronic, making it a viable treatment target (Johnson *et al*.,2018). This analysis confirmed a prior study (Albanwawy and Abdul-Lateef,2021), which found the *TviA* gene in 94.1% of *Salmonella typhi* isolates. Typhoid infection depends on the *Salmonella typhi*-only *TviA* gene. The protein-polysaccharide biosynthesis regulator *TviAVi* controls Vi-antigen, flagella, and Pilli formation. The isolate is Vi-positive because the pathogenic island SPI-7 of the *S. typhi* chromosome contains the *TviA* gene and encodes Vi-antigen (Liaquat *et al*.,2018).

According to the study, the Vi capsular antigen expression suppresses *S. typhi* infection in the hyperosmotic intestinal tract. This boosts bacterial invasiveness (Xie *et al*.,2010). In high osmolarity, *S. typhi* invades epithelial cells more but is less resistant to macrophage-mediated death. The Vi antigen helps macrophages survive and replicate while preventing invasion (Santander *et al*.,2008).

Conclusion

The study investigated the presence of *S. typhi*, the causative agent of typhoid fever, in gallbladder samples from patients with cholelithiasis in Erbil City, Iraq. The study found that *S. typhi* growth was 6.4%, with gallstones having the highest prevalence. Antibiotic susceptibility tests revealed resistance to commonly used antibiotics, while amikacin, azithromycin, tetracycline, levofloxacin, and ofloxacin showed better susceptibility. The study also revealed the virulence factors of *S. typhi* and the presence of typhoid toxin, contributing to the disease's severity. Further research is needed to understand the mechanisms behind *S. typhi's* persistence in the biliary tract and its potential role in gallstone development and related complications.

Conflict of interest

The authors declare that they have no conflict of interest.

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