

ORIGINAL ARTICLE

Molecular Detection of *Salmonella typhi* Isolated from Patients Undergoing Gallbladder Cholecystectomy in Baghdad

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SUMMARY

Background: *Salmonella typhi* is a specific strain of the *Salmonella* bacterium, responsible for triggering typhoid fever; a significant public health concern in developing nations.

Objective: The current study aimed to identify the bacteria from the gallbladder, taken during cholecystectomies of patients, by isolating *Salmonella typhi* and by using microscopic characteristics, biochemical and polymerase chain reaction (PCR) tests.

Methods: A total of 120 specimens were collected from the Baghdad Teaching Hospital, Iraq. A cross-sectional descriptive study was carried out from October, 2021, to July, 2022. During that study, 26 (54.2%) male patient tested positive for *Salmonella typhi* as well as 22 (45.8%) female patients. The age of the patients varied from < 30 to > 60 years. p-value > 0.05 was considered significant to confirm a relationship between age and *Salmonella typhi* effect for patients.

Results: Out of the 120 blood samples taken for this study, 48 (40%) tested positive by use of PCR test, 40 (33.3%) tested positive by use of the Widal test, 35 (29.1%) were positive for biopsy culture, and 35 (29.1%) were positive for blood culture. All *Salmonella typhi* isolates were found to be sensitive to the imipenem, cefepime, and ceftriaxone, but were resistant to gentamycin, ciprofloxacin, amikacin, erythromycin, and tetracycline (72%, 29%, 43%, 100%, 100%, respectively).

Conclusions: The real time polymerase chain reaction (RT-PCR) tests and the Vitek 2 compact system showed a high level of accuracy in the detection of *Salmonella typhi*. Multidrug resistance was observed, which should be a signal to reduce antibiotic consumption.

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KEYWORDS

gallbladder cholecystectomy, *Salmonella typhi*, molecular detection, Baghdad

LIST OF ABBREVIATIONS

RT-PCR - Real time-polymerase chain reaction
GD - Gallstone disease
GC - Gallbladder cancer
API-20E - Analytical Profile Index 20 Enterobacteriaceae
XLD - Xylose Lysine Deoxycholate Agar
TSI - Triple sugar iron
LF - Liver function test

UT - Urease test
IT - Indole test

INTRODUCTION

Salmonella typhi is a particular Salmonella type that causes typhoid fever and is considered a major public health problem, particularly in developing countries [1]. *Salmonella enterica* serovar typhi, also known as *Salmonella typhi*, only infects humans. *Salmonella typhi* motile, facultative anaerobe that is susceptible to various antibiotics. It contains different phenotypic characteristics, virulence features, and multi-drug resistance genes that complicate its treatment [2].

The gallbladder is a small, pouch-like organ in the upper right part of your tummy. It stores bile, a fluid produced by the liver that helps break down fatty foods. Adenocarcinoma, most common type of gallbladder cancer [3,4].

The ability of bile to aid in fat digestion does not play a role in its antimicrobial activity. An et al. [5], have stated: „Bile acids exhibit bacteriostatic and bactericidal effects against microbiota by disrupting their membranes, denaturing proteins, chelating iron and calcium, damaging DNA, and regulating host immunity via nuclear receptors such as farnesoid X receptor (FXR; NR1H4) and vitamin D receptor (VDR; NR1H1)” (*Salmonella*-Shigella and MacConkey agars) [4,5].

The Widal test is not able to identify *Salmonella enterica*. It is used for detecting infections indirectly rather than identifying microorganisms directly, while the polymerase chain reaction test has been shown to be both, quick and sensitive [6]. The current study is based on a comparative study that detected *Salmonella typhi* through ecological test and molecular methods. *FliC* gene encodes flagellin protein in bacteria (*Salmonella typhi*) and is used for identifying those bacterial infections [7].

The primary objective of this ongoing investigation is to isolate and identify *Salmonella typhi*, with an additional focus on utilizing PCR testing for identification and for the specific detection of *Salmonella typhi* patients.

MATERIALS AND METHODS

Study design

A cross-sectional descriptive study was carried out from October 2021 to July 2022 in Baghdad Teaching Hospital, Iraq, to detect *Salmonella typhi* among patients undergoing gallbladder cholecystectomies, suspected of having enteric fever.

Sample collection

A total of 120 different specimens were collected from the same patients (gallstones, gallbladder, sac samples, blood samples), who were undergoing a gallbladder cholecystectomy in Baghdad Teaching Hospital.

Isolation bacteria specimens

The isolation and identification of bacteria from specimens were carried out via cultural, morphological, and biochemical characteristics that were used for each isolated bacterial colony on Xylose Lysine Deoxycholate (XLD) Agar and blood agar and biochemical tests were also carried out. *Salmonella* was detected by Analytical Profile Index 20 Enterobacteriaceae (API-20E) systems and by the automated VITEK-2 compact system (VITEK-2 GNID kit). Widal testing was used for detecting O Ag and H Ag.

Antibiotic susceptibility Test

Isolates were tested for susceptibility to nine different antibiotic agents, including Imipenem, Ceftriaxone, Cefepime, Erythromycin, Ciprofloxacin, tetracycline, Amikacin, and Gentamycin (Oxoid, England).

Molecular diagnosis of *Salmonella typhi* isolates

The diagnostic system that was utilized was the *FliC* primers system, as it was applied by Arteta et al. [10], and was provided by Alpha DNA Company, as described.

flic (11)	F	5' ACT GCT AAA ACC ACT ACT 3'	363 bp
	R	5' TGG AGA CTT CGG TCG CGT AG 3'	

DNA purification

The main basic approach that is usually used for the purification of the DNA was performed according to the instruction manual of Promega, the supplier of the Kit.

Exclusion criteria

Patients that were on antimicrobial treatment within the past 3 days, patients with discharging ears of less than 3 weeks duration, and immunocompromised patients with chronic illnesses like diabetes mellitus, who had organ transplants, or who were on immunosuppressive drugs were excluded.

Ethical consideration

Ethical approval for conducting this study was requested from and approved by the College of Medicine, Baghdad, and the patients were informed about the study before collecting the specimens.

Statistical analysis

The SAS 2018 program was used to detect the effect of the different factors in this study. The chi-squared test was used for the comparison between the groups.

RESULTS

Salmonella is distinguished by a colony morphology which appears thick with irregular edges, by non-lactose fermentation, and by producing H₂S on an XLD agar. The *Salmonella typhi* appears as a Gram-negative coccobacillus. *Salmonella typhi* is positive for catalase and urease, for indole and VP negative, but positive for

Table 1. Distribution of the patients according to age group.

Age group	Number	Positive cases	Negative cases	$\chi^2 = 0.422$ $p \geq 0.05$ no significant relationship
20 ≥ years	5 (4.1%)	2 (40%)	3 (60%)	
20 - 29 years	23 (19.2%)	6 (26.1%)	17 (73.9%)	
30 - 39 years	30 (25%)	12 (40%)	18 (60%)	
40 - 49 years	24 (20%)	8 (33.3%)	16 (66.7%)	
50 - 59 years	20 (16.7%)	10 (50%)	10 (50%)	
≥ 60 years	18 (15%)	10 (55.6%)	8 (44.4%)	
Total	120 (100%)	48 (40%)	72 (60%)	

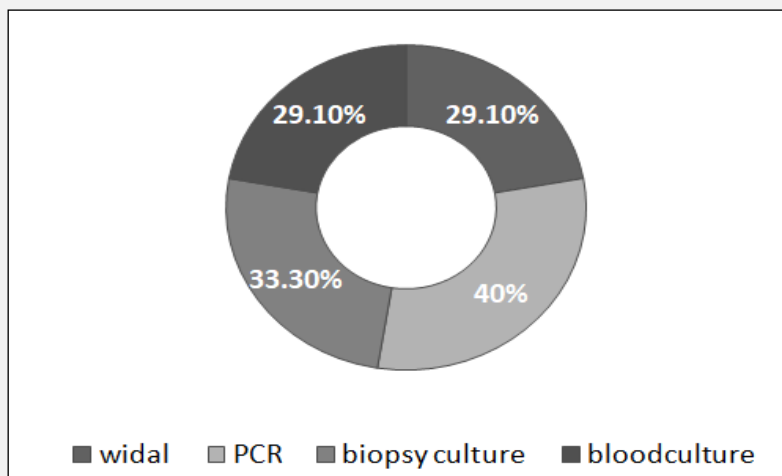


Figure 1. Identification of *Salmonella typhi* via different methods.

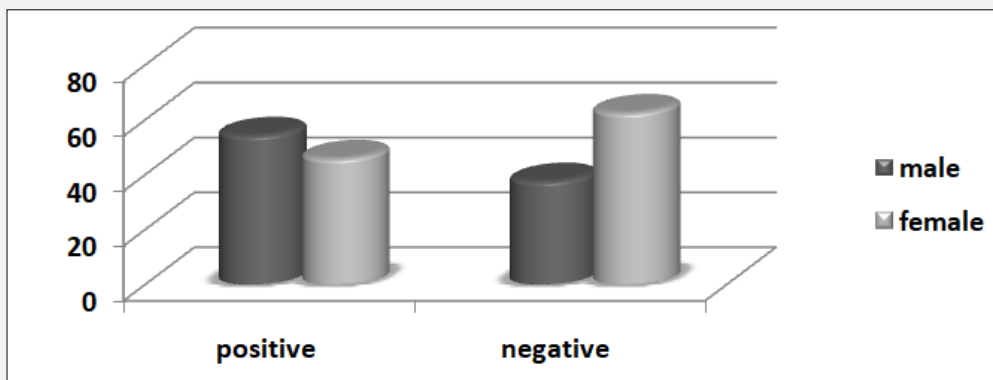


Figure 2. Gender's distribution of *Salmonella typhi* in gallbladder cholecystectomy patients.



Figure 3. PCR products of fil C gene, 599bp appear as positive results.

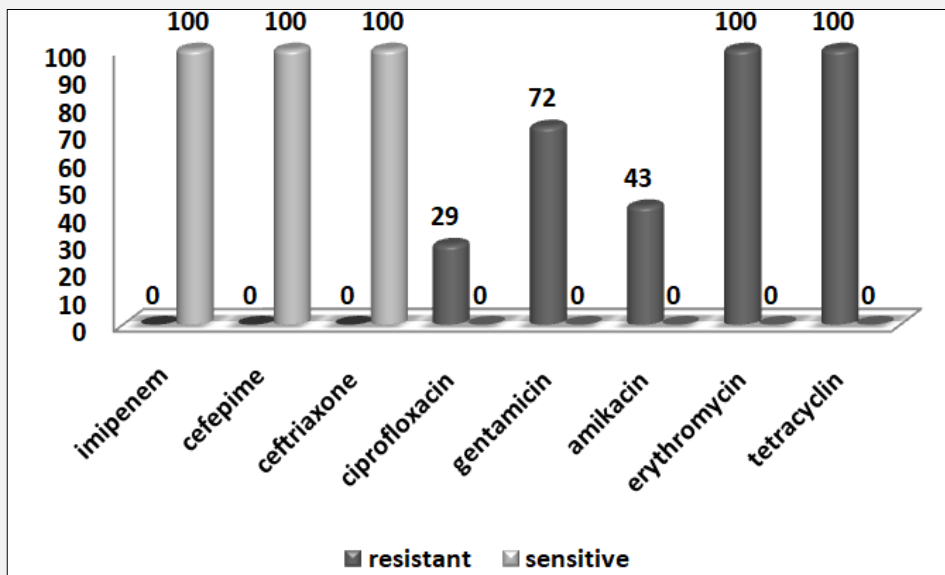


Figure 4. Resistance and sensitive's percentages of *Salmonella typhi* for some antibiotics.

methyl red, citrate utilization, and hydrogen sulfide. From the 120 blood samples taken from the typhoid patients, 48 (40%) showed positive results with PCR testing, while 40 (33.3%) had a positive Widal's test. Furthermore, 35 (29.1%) were positive for biopsy culture

and 35 (29.1%) were positive for blood culture, as shown in Figure 1.

Figure 2 shows the total numbers of *Salmonella typhi* cases that were examined and distributed among the male and female patients; 26 (54.2%) male patients test-

ed positive for *Salmonella typhi* and 22 (45.8%) female patients tested positive, $\chi^2 = 0.092$, $p > 0.05$.

Table 1 shows the age groups that were subjected to this study, ranging from < 30 to > 60 years. No significant relationship between age and *Salmonella typhi* infection were seen ($p \geq 0.05$).

Molecular detection of *Salmonella typhi* by PCR technique

Salmonella typhi were detected by PCR technique for amplification. The results revealed band, 599bp, for *FliC* gene as shown in Figure 3.

The antibiotic resistance profiles, as shown in Figure 4, reveal that all *Salmonella typhi* isolates are sensitive (100%) toward ceftriaxone, cefepime, and imipenem, while other *Salmonella typhi* isolates show resistance rates for ciprofloxacin (29%), for amikacin (43%), and for gen tamicin (72%). However, all *Salmonella typhi* isolates are resistant (100%) to tetracycline and erythromycin. The differences in sensitivity for the isolates are significant when $p < 0.05$, while there is no significant ($p \geq 0.05$) resistance of isolates towards ciprofloxacin and amikacin, as shown in Figure 4.

DISCUSSION

Despite a widespread agreement on the Widal's test sensitivity and specificity, the results of our investigation diverged from those of Andualem et al. [8], in which 11.7% of the *Salmonella typhi* isolates show positive test results and increases in the variation due to interpretational and methodological variation amongst the laboratories who were performing the same test. However, the outcomes of current research do not align with the researchers' findings of Arteta et al. [9].

Most serological tests for typhoid fever are accredited for use in hospitals and rely on traditional methods. These tests are involved in assessing the association between antigens and antibodies that present and appear in the patients' serum, in addition to determining the infection type; whether it is acute or chronic. The findings of this study align with the data reported in El-Tayeb et al., 2017 [10]; the study indicates that 58.33% of the *Salmonella* bacteria was included a 587bp genetic sequence. The same investigation was conducted previously by Kumar et al., 2012 [11]. The results of our current work matched with Mundher et al., 2023 [13]. Their study which used a 343bp reference size, making the two sets of findings consistent and reliable. The results from [13], who used a baseline size of 516bp, were consistent with our current findings as well.

All *Salmonella typhi* isolates were resistant to tetracycline and erythromycin with the highest rate (100%), while the resistance for ciprofloxacin, amikacin, and gentamicin was at only a low rate, and the resistance for imipenem, ceftriaxone, and cefepime was at zero percent. The resistance of *S. Typhi* isolates for these antimicrobial agents is clinically significant [14,15]. A

transferable drug resistance includes all antibiotics in common use, and transferring the drug resistance usually occurs *in vitro* and *in vivo* (metabolically normal), yet in a normal gut it can be inhibited by several factors such as anaerobic status, bile salts, and alkaline pH [16]. However, the transport occurs easily in the gut of people who are on oral antibiotic therapy, because of the selected pressure which is provided by the drugs. On the other hand, and due to the overuse of antibiotics in hospitals, it is said that "the hospital is a paradise for drug-resistant bacteria". Most of this concern is associated with the R-plasmid [17-19].

CONCLUSION

- 1) The use of the PCR tests and the Vitek 2 Compact system in detecting *Salmonella typhi* provides accurate results, which are very important for the diagnosis of typhoid.
- 2) Increases in the numbers of antibiotic-resistant *Salmonella typhi* are needed to raise the level of awareness of consuming antibiotics.
- 3) Continuous surveillance of the antibacterial sensitivity testing and a reasonable dosage of antibiotics should be maintained to avoid the outbreak of a multidrug resistance.

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Declaration of Interest:

No conflict of interest.

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