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Study *Salmonella typhi* in Cholelithiasis Patients in Related to Antibiotic Resistant and IL-37

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Abstract

Gallstones, also known as cholelithiasis, pose a significant threat to public health worldwide. While some people have recovered from typhoid fever, chronic infection with Salmonella typhi bacteria can lead to serious complications, including the development of gallstones and gallbladder cancer. This research investigated the epidemiology of *S. typhi*-infected gallstones related to interlukin-37. A cross-sectional study was conducted involving 125 cases of individuals aged 15 - 80 years, who visited the Erbil and Rizgari Teaching Hospitals and had their gallbladder removed. Gallbladder specimens and blood samples were collected to measure IL-37 using an ELISA assay. Additionally, an antibiogram test was performed. The study found that 101 patients (80.8%) cases had bacterial-infected cholelithiasis, while patients 24 (19.2%) did not show infection. Among these, 93 (74.4%) cases had other bacteria, while 8 (6.4%) cases had *S. typhi* in the gallbladder divided into gallstones 5 (4%), bile 1 (0.8%), and gallbladder tissue 2 (1.6%) cases. *S. typhi* forms biofilm on cholesterol gallstones more than pigment and mixed stones. The most infected age group 36–45 years were 32.8% cases, mostly females from urban areas. In 125 patients, the mean IL-37 level was 104.12 pg/ml compared with the control was 63.64 pg/ml. *S. typhi* was sensitive to piperacillin/tazobactam, ertapenem, meropenem, and fosfomycin, while resistant to another traditional antibiotic. In conclusion, *S. typhi* is more frequently found in gallstones than in the bile or gallbladder tissue in cholelithiasis patients, specifically in cholesterol-type gallstones. Most affected patients were females aged 36–45 years from urban areas. While *S. typhi* resistant to traditional antibiotics, it remains sensitive to ertapenem, imipenem, meropenem, and piperacillin/tazobactam. Elevated levels of IL-37 can reflect the presence of gallstones and indicate infected gallstones with *S. typhi*.

Keywords: gallstones, IL-37, *Salmonella typhi*, gallbladder, typhoid fever.

دراسة سالمونيلا تايفي في مرضى التحصي الصفراوي وعلاقته بمقاومة المضادات الحيوية وانترلوكين 37

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الخلاصة:

تشكل حصوات المرارة، المعروفة أيضًا باسم تحصي المرارة، تهديدًا كبيرًا للصحة العامة في جميع أنحاء العالم. وبينما تعافى بعض الأشخاص من حمى التيفوئيد، فإن العدوى المزمنة ببكتيريا السالمونيلا التيفية يمكن أن تؤدي إلى مضاعفات خطيرة، بما في ذلك تطور حصوات المرارة وسرطان المرارة. بحثت هذه الدراسة في التركيبة السكانية لحصوات المرارة المصابة ببكتيريا التيفية المرتبطة بالإنترلوكين 37. أجريت دراسة مقطعية شملت 125 حالة من الأفراد الذين تتراوح أعمارهم بين 15 و 80 عامًا، والذين زاروا مستشفيات أربيل ورزكاري التعليمية وأزيلت مرارتهم. تم جمع عينات المرارة وعينات الدم لقياس IL-37 باستخدام اختبار ELISA. بالإضافة إلى ذلك، تم إجراء اختبار المضادات الحيوية. وجدت الدراسة أن 101 مريضًا (80.8٪) لديهم حصوات صفراوية مصابة بالبكتيريا، في حين لم يظهر 24 مريضًا (19.2٪) عدوى. ومن بين هذه الحالات، كان لدى 93 حالة (74.4%) بكتيريا أخرى، بينما كان لدى 8 حالات (6.4%) *S. typhi* في المرارة مقسمة إلى حصوات المرارة 5 (4%)، والصفراء 1 (0.8%)، وأنسجة المرارة 2 (1.6%). تشكل *S. typhi* غشاء حيوي على حصوات المرارة الكوليسترولية أكثر من الحصوات الصبغية والمختلطة. كانت الفئة العمرية الأكثر إصابة 36-45 عامًا 32.8% من الحالات، معظمها من الإناث من المناطق الحضرية. في 125 مريضًا، كان متوسط مستوى IL-37 104.12 بيكو جرام / مل مقارنة بالمجموعة الضابطة التي كانت 63.64 بيكو جرام / مل. كانت *S. typhi* حساسة للبابيبرسيلين / تازوباكتام، وإيرتابينيم، وميروبينيم، وفوسفوميسين، بينما كانت مقاومة لمضاد حيوي تقليدي آخر. في الختام، تم العثور على *S. typhi* بشكل أكثر تكرارًا في حصوات المرارة مقارنة بأنسجة الصفراء أو المرارة لدى مرضى حصوات المرارة، وخاصة في حصوات المرارة من نوع الكوليسترول. كان معظم المرضى المصابين من الإناث في سن 36-45 عامًا من المناطق الحضرية. في حين أن *S. typhi* مقاومة للمضادات الحيوية التقليدية، إلا أنها تظل حساسة لإيرتابينيم وإيميبينيم وميروبينيم وبيبيراسيلين/تازوباكتام. يمكن أن تعكس المستويات المرتفعة من IL-37 وجود حصوات المرارة وتشير إلى حصوات المرارة المصابة بـ *S. typhi* .

Introduction:

Cholecystitis with gallstones is a common condition affecting the gastrointestinal and biliary systems, leading to significant morbidity and mortality. The formation of stones results in imbalances in the constituents of bile and in situations of biliary stasis, where bile is not flowing. Gallstones are usually classified as cholesterol stones or pigmented stones [1, 2]. Bacteria are implicated in approximately half of cases, regardless of gallstone presence or absence, and can result from any type of bacteria [3] . *Salmonella typhi* (*S. typhi*), is a well-known bacterium that infects the biliary tree in humans and produces typhoid fever [4]. These gram-negative bacilli bacteria, which are facultative anaerobes, part of the Enterobacteriaceae family, are resilient and can survive in dry environments for weeks and in water for months [5]. As a major zoonotic pathogen, *S. typhi* poses a global public health concern [6], causing over 115 million illnesses and 370,000 deaths annually [7]. The World Health Organization mentioned that one in ten people is infected with *S. typhi* [5, 8].

S. typhi possesses not only pathogenicity islands but also a unique genetic feature within the bacterial chromosome. Additional factors that enhance its pathogenicity like cytolethal distending toxins, flagella, and fimbria [9, 10]. The genome of *S. typhi* contains all the necessary genes for the bacterium to proliferate, travel throughout the host body, and elude the host's immune system to live inside the host cell are included within the genome of the chromosome [11]. *S. typhi* serotypes have different levels of pathogenicity and antibiotic resistance; this is due to virulence factors that are related to antibiotic resistance, which can help in the formulation of individual patient treatment plans [12]. Interleukin -37 (IL-37) primarily reduces innate and acquired immune responses by inhibiting the secretion of pro-inflammatory chemokine like IL-1β, IL-6 and TNF-α in macrophages and dendritic cells, thereby suppressing inflammation [13, 14]. IL-37 protects cells from apoptosis and necrosis

induced by various factors, including inflammation and oxidative stress and modulates the function of B cells, influencing their antibody production and immune response [15, 16]. Additionally, IL-37 promotes wound healing by stimulating tissue regeneration and angiogenesis, so it protects various organs like the liver, lungs, and kidneys from damage caused by inflammation and autoimmune diseases. IL-37 strongly inhibits lipopolysaccharide (LPS)-induced expression of IL-1α, IL-1β, tumor necrosis factor (TNF), IL-23, IL-17, IL-18, interferon-γ (IFN-γ) and chemokines (CC) including monocyte chemoattractant protein (MCP)-5 [17]. Moreover, studies have shown that plasma IL-37 levels are significantly elevated in patients with active disease compared with those with inactive disease and suggest that IL-37 expression level is closely related to the internal inflammatory state [18].
The current study aimed to investigate the role of Interleukin-37 in inflammation, as it has emerged as one of the most important inflammatory cytokines described in the last six years. A few of the studies done suggest that children with *S. pyogenes* infection have variations in immunological indexes that include IL-37 immunity gel and vitamin D compared to children without the infection. These findings suggest that these immunomarkers are involved in the pathogenesis of an infection with *S. pyogenes* [19].

Materials and Methods

1. Study design

The cross-sectional study was carried out in Erbil Governorate between December 10, 2023, and March 10, 2024.

2. Patients

The inclusion criteria for this study involved 125 patients with gallbladder disorders aged between 15 and 80 years in this study. Surgical operations were carried out at Erbil Teaching Hospital and Rizgari Teaching Hospital, while the gallstones were diagnosed using abdominal ultrasonography. Data regarding age, sex, place of residence, work, previous illness history, and disease signs were obtained using a survey.

3. Inclusion criteria

The present study included all the patients with Cholecystitis with gallstones.

4. Exclusion criteria

Individuals who had taken medications, such as antibiotics, for any infection within the previous four weeks were excluded from this study.

5. Control group

The participants in the comparison group were 80 healthy individuals in the age range of 15–80 years with no acute or chronic health conditions that could interfere with the research; they had been recruited from hospitals.

6. Collection of gallbladder samples for culture

The study involved collecting fresh gallbladder samples from patients who underwent cholecystectomy and dividing them into three groups: gallstone, bile, and tissue. These samples were transferred to sterile containers in a laboratory, and they inoculated onto *Salmonella-Shigella* (S-S agar) culture medium (Picture 4) for *Salmonella*-related infections.

6.1 Bile collection:

Bile was extracted from the gallbladder bile duct by using a sterile syringe or needle (Picture 1). Then transferred to sterile containers containing 5 ml of tryptone soy broth, and incubated at 37 °C for the duration of the day.

6.2 Gallstone collection:

During the surgery, gallstones were typically removed along with the gallbladder (Picture 1). The gallbladder was placed in a sterile screw tube with tryptone soy broth and cultured for 24 hours at 37 °C [20]. The various types, sizes, shapes, and colors of gallstones are shown in (Picture 2).

6.3 Gallbladder tissue collection:

During surgery, a small tissue sample from the gallbladder wall was collected, especially if there is suspicion of acute cholecystitis (Picture 1). In sterile tubes filled with brain-heart infusion broth, the tissue was cultured for 24 hours at 37 °C [21].

6.4 Bacterial culture:

After overnight incubation in enrichment broth, bacterial culturing of the three samples (bile, gallstone, and gallbladder tissue) was prepared. Gallbladder specimen swabs were inoculated on Petri plates containing S-S agar and cultured for 24 to 48 hours at 37 °C (Picture 4). The study examined colonies morphologically and microscopically, identifying microorganisms in a pure culture based on physical traits. Bacteria isolated from bile, gallstones, and gallbladder tissue were verified using the VITEK-2 compact system, following the manufacturer's instructions.

7. Blood collection

Blood samples were collected from both patients and control. The blood was allowed to clot at 37^{O}C/30 minutes, then centrifuged for 15 minutes at 3000 rpm after that it was put in Eppendroff tubes which were kept at -20 ^{O}C till used by ELISA method for measuring IL-37 level antibodies.

Determination of human interleukin -37 by ELISA

The ELISA sandwich kit (Human Interleukin 37 ELISA kit, Elabscience, USA) was utilized in this study. The micro-ELISA plate provided in this kit had been pre-coated with an antibody specific to human IL-37. Samples (or standards) were added to the micro-ELISA plate wells, and combined with the specific antibody. Then a biotinylated detection antibody specific for human IL-37 and avidin-horseradish peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. A substrate solution was added to each well, resulting in a blue color only in those wells that contained human IL-37, the biotinylated detection antibody and avidin-HRP conjugate. The enzyme-substrate reaction is terminated by the addition of a stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of human IL-37 (Picture 7).

Antibiotics susceptibility test by Vitek-2 system

Antibiogram testing was performed with the automated VITEK-2 compact system based on MIC technique determination using AST- N417 cards (Picture 5). The following antibiotics were tested: Amoxicillin/Clavulanic Acid, Piperacillin/Tazobactam, Cefazolin, Cefuroxime, Cefuroxime Axetil, Ceftazidime, Ceftriaxone, Cefepime, Ertapenem, Imipenem, Meropenem, Amikacin, Gentamicin, Ciprofloxacin, Fosfomycin, Nitrofurantoin and Trimethoprim/ Sulfamethoxazole [22] as in (Picture 5).

The Procedure was done according to (Picture 6) [23]**:**

1. The 0.5 McFarland bacterial suspension was diluted to $1.5x10^{7}$ CFU/ml DensiCHEK plus meter in 0.45% saline.

2. Remove the AST card from its package and place it into the designated slot on the Vitek-2 instrument.
3. Using a calibrated pipette, dispense the prepared inoculum into the designated wells on the AST card, as per the manufacturer's instructions. Each well contains a different antibiotic at specific concentrations.
4. Close the card with the provided seal and place it back into the Vitek-2 instrument.
5. Select the appropriate test profile for *S. typhi* on the Vitek-2 software. The instrument will automatically incubate the card and measure bacterial growth in each well over time.
6. After several hours, the Vitek-2 software will analyze the growth data and generate a report showing the minimum inhibitory concentration (MIC) of each antibiotic for the *S. typhi* isolate. The MIC is the lowest concentration of the antibiotic that inhibits visible bacterial growth.

Ethical approval

The study adhered to the Declaration of Helsinki's guidelines for conducting research with minimal risk to participants. Before collecting any samples, the researchers ensured that patients provided both verbal and written consent. As indicated in Document 119 (dated 16/3/2022), the research protocol, participant information, and consent form were reviewed and approved by a local ethics committee.

Statistical analysis:

The study included data of 125 patients with cholelithiasis and 80 healthy control persons. The chi-square test and T-test were used. P- value ≤ 0.05 was considered significant, while greater than 0.05 was considered non-significant

Results:

1. Frequency of cholelithiasis patients according to age and sex

Table 1 presents the age and sex distribution of cholelithiasis patients. The most affected age group was 36 to 45 years, with approximately 41 cases (32.8%) being female, followed by 35 (28.2%) cases aged 26–35 years, also females more than males. A significant association was found between age, sex, and cholelithiasis, indicating a significant variation in patient distribution across age groups.

Table 1: Distribution of patient and control group according to age and sex

Age (years)	Patient group				Control group			
	NO.	%	Male	Female	NO.	%	Male	Female
15 – 25	20	16	4	16	13	16.25	1	12
26 - 35	35	28	8	27	20	25	2	18
36 - 45	41	32.8	6	35	25	31.25	9	16
46 - 55	19	15.2	4	15	14	17.5	6	8
56 - 65	6	4.8	2	4	7	8.75	5	2
66 - 75	3	2.4	3	0	1	1.25	1	0
> 76	1	0.8	1	0	0	0	0	0
Total	**125**	**100**	**28**	**97**	**80**	**100**	**24**	**56**
Chi squared test	15.78 (0.014 *S)				18.48 (0.005 **HS)			

*S: Significant difference between groups (p-value < 0.05)
**HS: High significant difference between groups (p-value < 0.01)

2. Distribution of cholelithiasis patients according to residence

Table 2 shows the residence distribution of patients and the control group, revealing that 88 patients (70.4%) were from urban areas, while 37 patients (29.6%) were from rural areas.

The control group had 63 (78.75%) urban cases and 17 (21.25%) rural cases, with no significant difference in residence.

Table 2: Distribution of cholelithiasis patients and control group according to residence

Residence	Patient group		Control group		Chi-square test (p-value)
	No.	%	No.	%	
Urban	88	70.4	63	78.75	1.75 (0.185 *NS)
Rural	37	29.6	17	21.25	
Total	**125**	**100**	**80**	**100**	

*NS: No significant difference between groups (P > 0.05)

3. Isolation of bacteria from different site of gallbladder

The review included 125 patients with gallbladder infections who suffered from signs and symptoms of gallbladder inflammation or stones which were confirmed by ultrasound and investigations. These patients underwent cholecystectomy, which was divided into three types (75 gallstones, 35 bile, and 15 gallbladder). Statistical analysis using (The chi-square test 2.809 and p-value 0.590) indicated no significant association between gallbladder type and bacterial growth, with 101 (80.8%) of samples showing bacterial growth and 24 (19.2%) showing no bacterial growth, as shown in (Table 3).

Table 3: Distribution of bacterial growth in cholelithiasis patients

Type of gallbladder Sample	Gallbladder sample		Bacterial growth		No bacterial growth		Chi-square test (p value)
	No.	%	No.	%	No.	%	
Gallstone	75	60	57	45.6	18	14.4	2.809 (0.590 *NS)
Bile	35	28	31	24.8	4	3.2	
Gallbladder tissue	15	12	13	10.4	2	1.6	
Total	**125**	**100**	**101**	**80.8**	**24**	**19.2**	

*NS: No significant difference between groups (P > 0.05)

4. *S. typhi* growth distribution according to sources in the gallbladder

The analysis of 101 bacteria isolates from gallstone, bile, and gallbladder tissue indicated that gallstones were the most conducive environment for the growth of bacteria, with a microbial culture positivity rate of 45.6%, 24.8%, and 10.4%, respectively (Table 4).

The current review showed that among 125 specimens of gallbladder from gallbladder disease (GD) patients, only 8 (7.92%) samples tested positive for *S. typhi*. Specifically, of the 57 samples with bacterial growth, 5 (4.95%) exhibited *S. typhi* growth. For the bile type, out of the 31 samples with bacterial growth, 1 (0.99%) showed *S. typhi* growth. For the gallbladder tissue type, out of the 13 samples with bacterial growth, 2 (1.98%) showed *S. typhi* growth. The chi-square test yielded a p-value of 0.738, meaning there was no significant difference (NS) between the groups.

Table 4: *S. typhi* growth distribution according to sources in the gallbladder

Type of gallbladder sample	Bacterial growth		Other *S. typhi* growth		*S. typhi* growth		Chi-square test (p value)
	No.	%	No.	%	No.	%	
Gallstone	57	45.6	52	51.49	5	4.95	1.98 (0.738 *NS)
Bile	31	24.8	30	29.70	1	0.99	
Gallbladder tissue	13	10.4	11	10.89	2	1.98	
Total	**101**	**80.8**	**93**	**92.08**	**8**	**7.92**	

*NS: Non-significant difference between groups (P > 0.05)

5. Interleukin-37 in cholelithiasis patients and control:

Table 5 shows the interleukin-37 levels in cholelithiasis patients compared to the control group. The mean IL-37 level in patient groups was 104.12 pg/ml, while the levels of IL-37 were 63.64 pg/ml in control groups. There was a highly significant difference between the IL-37 levels in the patient and control groups (p-value < 0.001 by using T-test).

Table 5: Interleukin-37 level in patients' group and control group

Studied group	No.	IL – 37 (Pg/ml)		T-test (p-value) DF
		Mean	SD	
Patient group	125	104.12	19.52	16.76 (0.001 *HS) 203
Control group	80	63.64	11.51	

*HS: Highly significant difference between groups

6. Antibiotic sensitivity test:

Table 6 displays the results of the antibiotic sensitivity test for *S. typhi* from cholelithiasis patients, conducted using the Vitek-2 compact system (as previously described in the materials and methods section). The table presents the susceptibility, resistance, and intermediate susceptibility percentages, shown as follows:

Table 6: Antibiotics susceptibility test for *S. typhi* isolates by Vitek-2 compact system (Number of *S. typhi* = 8)

No.	Antibiotics	MIC (µg /ml)	Susceptible strains No. (%)	Intermediate resistant strains No. (%)	Resistant strains No. (%)
1	Amoxicillin/Clavulanic Acid	<= 2,8	6 (75%)	0	2 (25%)
2	Piperacillin/Tazobactam	<= 4, 8	7(87.5%)	0	1(12.5%)
3	Cefazolin	>= 64	1(12.5%)	0	7(87.5%)
4	Cefuroxime	>= 64	1(12.5%)	0	7(87.5%)
5	Cefuroxime Axetil	>= 64	0	1(12.5%)	7(87.5%)
6	Ceftazidime	>= 64,32	3(37.5%)	0	5(62.5%)
7	Ceftriaxone	>= 64	2(25%)	0	6(75%)
8	Cefepime	4,8	2(25%)	3(37.5%)	3(37.5%)
9	Ertapenem	<= 0.12	7(87.5%)	0	1(12.5%)
10	Imipenem	<= 0.25	6(75%)	1(12.5%)	1(12.5%)
11	Meropenem	<= 0.25	7(87.5%)	0	1(12.5%)
12	Amikacin	<= 1	6(75%)	0	2(25%)
13	Gentamicin	<= 1	6(75%)	0	2(25%)
14	Ciprofloxacin	>= 4	2(25%)	0	6(75%)
15	Fosfomycin	<=16	7(87.5%)	0	1(12.5%)
16	Nitrofurantoin	<=16	6(75%)	0	2(25%)
17	Trimethoprim/ Sulfamethoxazole	>= 320,<=20	3(37.5%)	0	5(62.5%)
	Chi squared test (p value)		**83.88 (0.0000015 *VHS)**		

*VHS: Very highly significant difference between groups (p-value < 0.0001)

Discussion

Consistent with a recent study, Gram-negative bacteria accounted for the primary cause of gallbladder infection [24], comprising 101 (80.8%), which establishes a rate of

80.49%. Faecal coliforms are bacteria that are classified as belonging to the Enterobacteriaceae family. Early colonization of the digestive tract by enteric microbiota, which may be found in contaminated meat, vegetables, water, and soil, can lead to the development of cholelithiasis by blending in with the microflora.
These findings might be attributed to the ability of *S. typhi* to create bile salt enzymes, phospholipase and beta-glucuronidase, which are crucial for the development of brown pigment stones [25]. These enzymes break down bilirubin diglucuronide in bile to produce free, unconjugated bilirubin linked to calcium. Bacterial bilirubin and calcium palmitate are deposited when the bile's pH is low [26]. Additionally, the glycocalyx capsule of *S. typhi* enables it to adhere to calcium bilirubin solid pigment, collecting solid dye particles and placing them in the stone nucleus [27].
Gallstones can harbour *Salmonella typhi*, leading to chronic inflammation due to the increased colonisation surface area. Conventional antibiotics are difficult to eradicate [4], and when inside the biofilm, *S. typhi* can alter gene expression and produce a slime covering stone surfaces, making it difficult to eradicate [8].

This study found that cholesterol gallstones (11.11%) are particularly prone to *Salmonella typhi* biofilm formation due to their composition of cholesterol, lipids, and bile pigments, which provides a favorable surface for adhesion and biofilm development. Additionally, the hydrophobic nature of cholesterol may protect the biofilms from abrasive bile and immune system attacks [8].

Frequency of isolated bacteria from different samples of gallbladder

Out of 101 bacterial isolates obtained from gallbladder samples, 45.6% showed growth in gallstones, 24.8% in bile, and 10.4% in gallbladder tissue. *S. typhi* was identified in approximately five cases in gallstones, 2 cases in gall bladder epithelial tissues, and one case in bile samples. These results agree with those of a previous investigation by Mansour *et al*. [28], which found approximately 28, 12, and 4 cases of *S. typhi* in gallstones, gall bladder epithelial tissues, and bile samples, respectively.

Frequency of cholelithiasis patients according to age and sex

The study found that infection occurs in both sexes, in women (77.6%) than men (22.4%) due to female sex hormones. The sex ratio is approximately 1:3.5. This ratio agrees with other studies: Manan *et al.* [29] reported a ratio of about 1:3.85, Thapa *et al.* [30] reported a ratio of about 1:3, and Ghazal *et al*. [31] reported a sex ratio of 1:4 males to females.
The findings of this study revealed that women have a higher incidence of gallstones than men, possibly due to factors such as female sex hormones, reproduction, and the number of children they have. Gallstones are more prevalent in women and typically develop beyond 40 years [32, 33].

The study confirms that gallbladder inflammation affects all ages, with a 32.8% increase in the 36–45 age group, consistent with a previous study of 30% by Ali, *et al.* [34].
Gallbladder issues are rare in individuals under ten years old, however, as people age, their frequency increases, and gallstones are 4–10 times more common in the elderly. While they may develop at any age, gallstones usually start to cause issues in the forties, fifties, or sixties. Doctors are warned against doing surgery because of the potential for medication exposure and its consequences [35] .
Gallstone formation increases with age due to alterations in bile composition, such as elevated cholesterol saturation decreased bile salt synthesis, and declines in gallbladder function [36]. The changes in the hormones especially in the women who had multi-parity and underlying medical disorders like obesity, diabetes, and metabolic syndrome can further

contribute to gallstone development. These changes can be more likely when combined with other factors like the use of drugs, the amount and type of foods taken, and genetic predisposition, which are more probable as one grows older [37].

Frequency of cholelithiasis patients according to residence

In this study, 88 patients (70.4%) with gallbladders were from cities, while 37 (29.6%) were from rural areas. This trend can be attributed to urban diets characterized by high intakes of sugar, unhealthy fats, and processed carbohydrates, as well as low intakes of fiber, fruits, and vegetables. These factors can affect the content of bile and cholesterol production, and this may lead to the formation of gallstones. Rural diets that include fruits, grains, and legumes help improve digestion, thus reducing the risk of getting gallstones [38]. Additionally, obesity is more common in cities, increasing the chances of developing gallstones, and limited physical exercise may cause poor gallbladder contractions [39, 40].

Antibiotic susceptibility (Picture 6)

The multi-drug resistance of the *S. typhi* isolates was validated by the antibiotic susceptibility test conducted on the Vitek-2 compact system; this conclusion was backed by Al-Aaragy, *et al*. and Al-Quraishi [41, 42].

The results of this study indicated that *S. typhi* isolates are pan-sensitive to amikacin, ertapenem, imipenem, meropenem, piperacillin/tazobactam, gentamycin, and amoxicillin/clavulanic acid. These results pertain to the study by Al-Quraishi [42], and are resistant to cefazolin, cefuroxime, ceftriaxone, ceftazidime, and cefepime, which aligns with previous research by Hanan *et al*. and Abd El-Aziz *et al.* [43, 44].

The study found a high prevalence of antibiotic resistance among bacterial isolates, possibly due to over-prescription, improper administration, or over-the-counter sales. This resistance may be influenced by the selection pressure, which is exerted by commonly used antibiotics on circulating microorganisms. Virulence factors and gene mutations contribute to resistance in gastrointestinal infections, including aggression, abuse, antimicrobial efficiency, and control. Incomplete or inappropriate antibiotic treatment regimens also contribute to drug resistance.

Level of IL-37 in cholelithiasis patients

Our findings indicated that cholelithiasis patients exhibited higher serum IL-37 levels compared to the healthy control group. The IL-37 protein, which is essential for controlling inflammation and inhibiting the generation of pro-inflammatory cytokines, may be up-regulated by pro-inflammatory cytokines and inflammatory stimuli [15, 45].

Studies have shown the presence of the anti-inflammatory cytokine IL-37 in several tissues, including the uterus, lymph nodes, and liver. The administration of lipopolysaccharide decreases the synthesis of chemokines and inflammatory cytokines, including TNF, IL-23, IL-17, and IL-18 [46].

Research indicates that the timely synthesis of IL-37 by the host immune system averts excessive tissue damage caused by microbial infections.

Conclusion

Based on the results, we conclude that patients with gallbladder disease (cholelithiasis) exhibited bacterial growth in the gallbladder, especially from Gram-negative bacteria such as *S. typhi,* which were found in gallstones other than bile and gallbladder tissue. Cholesterol stones were identified as the most common infected type. Women in the age group of 36-45 years who were suffering from cholelithiasis had *S. typhi* infection with a higher level of IL-37 than control, which was important in the regulation of inflammation by inhibiting the production of pro-inflammatory cytokines. *S. typhi* was resistant to traditional antibiotics

such as cefazolin and cefuroxime but sensitive to ertapenem, imipenem, meropenem, and piperacillin/tazobactam.

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Authors' Declaration

Conflicts of Interest: None.
As a result, we attest that each figure and table in the book is authentic. Moreover, permission had been given for the reprinting of the figures and photos, which are attached to the text but are not mine or ours.
Ethical Clearance: The ethical committees of Tikrit University, Erbil Teaching Hospital, and Rizgari Teaching Hospital gave their approval to the study.

Authors' contributions:

Study design by Alaa Z.R. Amer H.M., analysed by Alaa Z.R. and Amer H.M. acquired the data, and all authors approved the final text after their contributions to interpretation and correction were made.

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Appendex

Picture 1: Collect the bile and gallstone from gallbladder

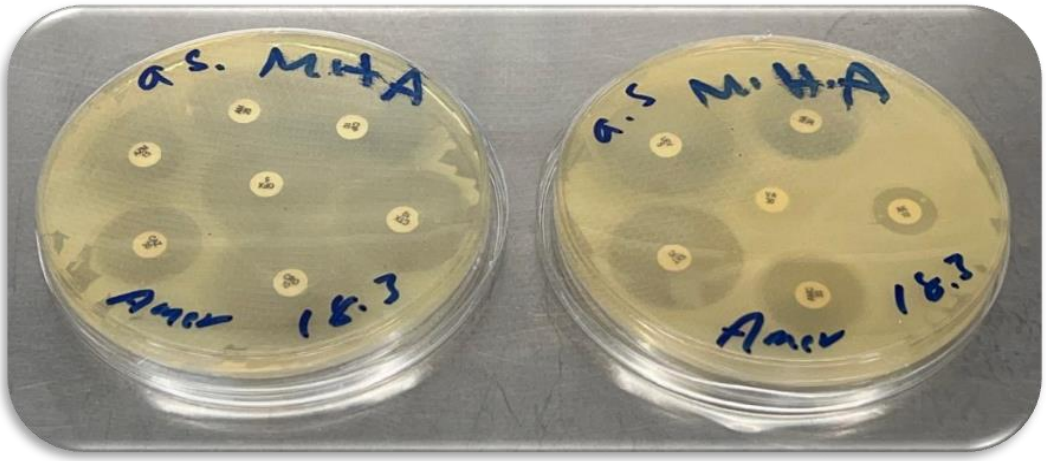
A **B** **C**

Picture 2: Type of gallstones. A: Pigment stone, B: Cholesterol stone, C: Mixed stone

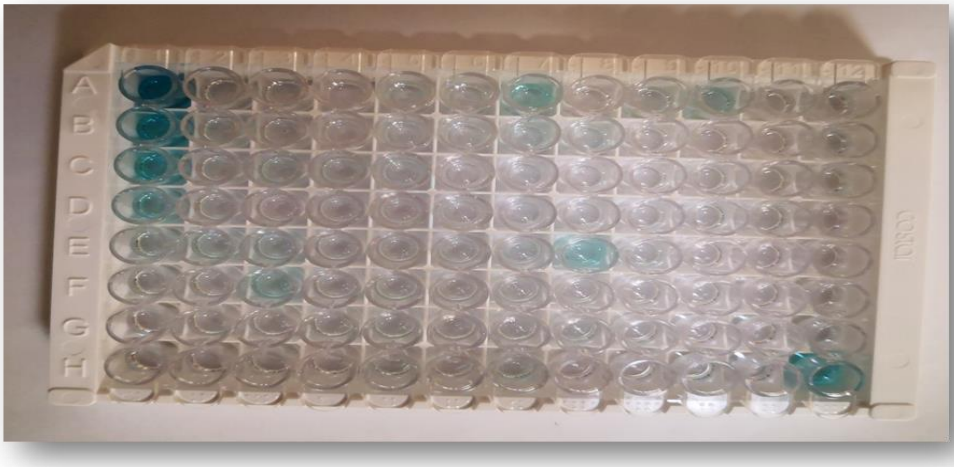
Picture 3: The gallstones differ in shapes, colures and sizes they are ranging from 2 mm - 2 cm.

Picture 4: *Salmonella typhi* on S.S agar analysis AST

Picture 5: Cassette with VITEK2 card for



Picture 6: Antibiotic sensitivity test (AST) of *S. typhi* by Vitek-2 Compact System



Picture 7: Reading the result of Interleukin – 37 level by ELISA reader