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HISTOPATHOLOGICAL AND MICROBIAL SPECTRUM CORRELATES IN GALLSTONE DISEASE

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ABSTRACT

Background: Cholelithiasis is a very common surgical problem that is often accompanied by chronic inflammation of the gallbladder and may be complicated by bacterial colonization. It is not fully understood how much infection is involved in the development of gallstones. **Objectives:** Identification of bacterial infection and its characterization in the gall bladder of gall stone patients; to find the spectrum of the pathogens in both microbiological and histopathological approach. **Materials and Methods:** A cross sectional observational study was done on 100 patients who underwent elective laparoscopic cholecystectomy during the period from 1st September 2025 to 1st March 2026 in Medical City Hospital, Baghdad, Iraq. Gall bladder samples were taken intraoperatively and cultured at various sites in the laboratory, and histopathology was performed by hematoxylin and eosin staining. **Results:** The female-to-male ratio was 5.0:1, with a mean age of 56.8 ± 1.4 years. Bacterial cultures were positive for 88/100 patients (88%) and negative (no growth) in 12/100 (12%). The most common organism found was Escherichia coli (34.1%), followed by Enterobacter aerogenes. The overall incidence of histopathological abnormalities was significantly higher in the gallbladders containing calculi (95%) than in those without calculi ($P < 0.05$). Histological lesions that were most frequently observed were Rokitansky–Aschoff sinuses. **Conclusions:** E. coli is a common bacteria that is highly prevalent in patients with cholelithiasis and can play a role in the disease pathogenesis. Findings endorse the probable necessity of multi-spectrum antimicrobial approaches in certain situations. Advanced molecular techniques should be used in future studies to further clarify the involvement of bacteria in gallstone formation.

KEYWORDS: Cholelithiasis, Bacterial infection, Escherichia coli, Histopathology, Rokitansky-Aschoff sinuses.

INTRODUCTION

Cholelithiasis is one of the most common hepatobiliary diseases worldwide and is a significant indication for surgical intervention, especially in the form of laparoscopic cholecystectomy.[1,2] Although there has been much research, the exact mechanisms involved in gallstone formation are poorly understood and increasing interest has focused on the possible role of microbial factors in these mechanisms.[3] This is caused by gallstones forming in the gallbladder due to a complex interplay between the composition of bile, motility of the gall bladder, and the function of the mucosa.[4] Traditionally, bile has been considered a sterile biological fluid under physiological conditions. But, this notion is undermined by recent evidence that bacterial colonization of the biliary system is far more common than previously thought, especially in patients with gallstone disease.[5,6] The presence of microbes has been identified in bile, in gallbladder mucosa, and even in the center of gallstones, which suggests crucial questions about their relationship in triggering and development of cholelithiasis. Of these, gram-negative bacteria of the enteric type, especially Escherichia coli, have been consistently linked because they are able to survive in bile and have the ability to generate enzymes such as β-glucuronosidase that may facilitate pigment stone formation.[7]

Bacteria could play a role in gallstone pathogenesis in a variety of ways from a mechanistic standpoint. These

consist of enzymatic changes in bile components, increased cholesterol crystallization and biofilm formation to provide a nucleating scaffold. In addition, chronic bacterial presence can cause chronic low-grade inflammation of the gall bladder wall, which can cause structural and functional changes and increase the risk of bile stasis and stone formation.[8,9] Infection, inflammation and biliary dynamics, all of which play a multifactorial role in the disease[10], are all involved in this.

The chronicity and severity of gallstone disease can be determined from histopathologic changes in the gallbladder. Mucosal hyperplasia, inflammatory cell infiltration, fibrosis and Rokitansky–Aschoff sinuses are common features and indicate adaptive and pathological responses to chronic irritation and inflammation. In particular, these sinuses are believed to be mucosal invaginations into the muscular layer, which could allow bacterial persistence and entrapment. They are often seen in patients with chronic cholecystitis, and may therefore play a role in both the disease course and its pathobiology.[11-15]

The association between microbial colonization and histopathological changes, however, has not been well characterized, especially in developing countries where environmental, dietary, and health care conditions may affect disease patterns.[16,17] The majority of the published work has concentrated on microbiological profiling or histological evaluation alone and fewer on both types of evaluation in the same population. One important gap in this understanding is the lack of detailed information on how the presence of bacteria is linked to structural changes in the gallbladder and how this is related to the formation of gallstones.[18]

Also, geographic differences in the profile of microbial flora and resistance to antibiotics require a region-specific investigation for guiding clinical management of the disease.[19] Within the context of delayed presentation and inadequate access to health care in such environments, chronic gallbladder disease could have unique microbiological and pathological characteristics. Hence, studies conducted in these settings are also critical to provide context-specific evidence to support surgical and antimicrobial decision making.[20,21]

Considering these facts, the present study is designed to give an integrated assessment of the bacterial colonization and histopathological changes in cholelithiasis patients' gall bladder. This study aims to help shed light on the possible relation between infection and histopathological changes of gallstones, in addition to microbiological culture techniques, in a tertiary health care centre in Iraq.

MATERIALS AND METHODS

Study Design and Setting

This is a cross-sectional observational study carried out during six months from September 2025 to March 2026 in Medical City Teaching Hospital, Baghdad, Iraq, a tertiary level hospital. The study aimed to assess the microbiological and histopathological features of the gallbladder tissue taken from the patient with cholelithiasis during surgery.

Study Population

100 consecutive patients with cholelithiasis were enrolled for elective laparoscopic cholecystectomy. All patients were recruited regardless of gender, and ranged in age between 20 and 78 years.

Inclusion Criteria

- Patients with ultrasonographically confirmed cholelithiasis
- Age ≥18 years
- Patients undergoing elective laparoscopic cholecystectomy
- Patients who provided informed consent

Exclusion Criteria

- Patients receiving antibiotic therapy within two weeks prior to surgery
- Patients with known malignancy of the hepatobiliary system
- Inadequate or contaminated specimens
- Patients with acute cholecystitis requiring emergency intervention

Sample Collection and Handling

Gallbladder specimens were then removed from the patient and placed in sterile conditions in the laboratory immediately after their surgical removal. Both gallbladders were opened with sterile instruments in a longitudinal fashion. To minimize sampling bias, bile and mucosal swabs were obtained from several areas, such as the fundus, body, and neck.

Sterile containers were used to collect specimens which were processed immediately to prevent contamination or overgrowth by bacteria. The wall of the gallbladder was preserved in 10% neutral buffered formalin in parallel in order to perform histopathological examination.

Microbiological Analysis

Collected samples were cultured on standard culture media such as blood agar and MacConkey agar and were incubated at 37°C under aerobic conditions for 24–48 hours. Bacteria were identified using standard biochemical tests and Gram staining and based on the shape of the colonies.

Polymicrobial growth was present when two or more bacterial species were isolated from the same specimen. The culture-negative samples were made up after 48 hours of culture with no sign of growth.

Histopathological Examination
Specimens were routinely formalin fixed, embedded in paraffin, cut in 4-5 µm thickness and stained with hematoxylin and eosin (H&E). The light microscopy was performed on the microscopic level to assess structural and inflammatory changes.

Histopathological parameters assessed included.

- Presence of Rokitansky–Aschoff sinuses
- Mucosal hyperplasia
- Inflammatory cell infiltration
- Fibrosis
- Other degenerative or reactive changes

All slides were reviewed independently to ensure diagnostic consistency.

Statistical Analysis
Data were analyzed with the help of the statistical software (IBM SPSS Statistics for Windows, Version 26.0, IBM Corp., Armonk, NY, USA). Data for continuous variables were represented as mean ± standard deviation (SD) and for categorical variables as frequencies and percentages. The Chi-square (χ^2) test or Fisher's exact test was used for comparison of categorical variables, as appropriate. Odds ratios (OR) and 95% confidence intervals (CI) were used to evaluate the association. P value < 0.05 was regarded as statistically significant.

The Chi-square (χ_2) test or Fisher's exact test were used for comparisons between groups as appropriate. The strength of association was evaluated using odds ratios (OR) with 95% confidence intervals (CI). The p value of < 0.05 was accepted as statistically significant.

Ethical Considerations
The study protocol was approved by the institutional ethics committee of Medical City teaching hospital. All participants consented to be included after signing an informed consent form. All procedures were performed in compliance with the ethical standards for biomedical research and with the guidelines of the Declaration of Helsinki.

RESULTS

1. Demographic Characteristics
There were 100 patients operated for elective laparoscopic cholecystectomy due to cholelithiasis. There was a strong female predominance in the study population with 83 females (83%) to 17 males (17%) (5.0:1 female to male ratio).

The age of patients ranged from 20 to 78 years, with a mean age of 56.8 ± 1.4 years (95% CI: 54.2–59.4). The majority of patients (64%) were 50 years old or over, as there is a higher prevalence of gallstone disease in older people.

Table 1: Demographic characteristics.

Variable	Value
Total patients	100
Females	83 (83%)
Males	17 (17%)
Female:Male ratio	5.0:1
Age range (years)	20–78
Mean age ± SD	56.8 ± 1.4
Age ≥50 years	64 (64%)

2. Microbiological Findings
A total of 88 patients had positive bacterial culture results (88%, 95% CI: 81.7%–94.3%), while 12 patients (12%) had no bacterial culture results.

Escherichia coli (30 cases; 34.1%) was the most common organism isolated from the culture positive samples (n=88), followed by Enterobacter aerogenes (19 cases; 21.6%). Isolates of Klebsiella spp., Pseudomonas aeruginosa and Staphylococcus aureus were also found in isolation.

Polymicrobial growth was found in 22 samples (25% of culture positive samples); this means there was mixed bacterial colonization.

Table 2: Microbial spectrum in gallbladder samples.

Organism	Frequency (n=88)	Percentage (%)
Escherichia coli	30	34.1%
Enterobacter aerogenes	19	21.6%
Klebsiella spp.	15	17.0%
Pseudomonas aeruginosa	14	15.9%
Staphylococcus aureus	10	11.4%

3. Histopathological Findings
The abnormalities were seen in 94 patients (94%) and in 6 cases (6%) no significant changes were identified through histopathology.

The most common histological finding was Rokitansky–Aschoff sinuses (RAS), which was present in 66 cases (66%). The chronic inflammatory infiltration was found in 62 cases (62%), mucosal hyperplasia was found in 55 cases (55%), and fibrosis was found in 48 cases (48%).

There were significantly less pathological changes observed in Acalculous gallbladders (n=4) compared to Calculous cases (P< 0.001).

Table 3: Histopathological alterations.

Histopathological feature	Frequency (n=100)	Percentage (%)
Rokitansky–Aschoff sinuses	66	66%
Chronic inflammation	62	62%
Mucosal hyperplasia	55	55%
Fibrosis	48	48%
No significant changes	6	6%

4. Association Between Infection and Histopathology

There was a significant correlation between the positive bacterial culture and histopathological abnormalities (P = 0.002).

The odds of having advanced histopathological changes were greater among culture-positive patients than among culture-negative patients (OR = 8.9; 95% CI = 2.1–37.4).

Table 4: Association between culture positivity and histopathology.

Group	Histopathology (+)	Histopathology (−)	P-value	OR (95% CI)
Culture positive (n=88)	84	4	0.002	8.9 (2.1–37.4)
Culture negative (n=12)	10	2	—	—

5. Calculous vs Acalculous Gallbladder Disease

There were 96 patients with calculous cholecystitis and 4 with acalculous gallbladder disease.

There was significant difference between the incidence of histopathological abnormalities in calculous cases (94/96; 97.9%) and acalculous cases (1/4; 25%) (P < 0.001).

In the same way, the cultured bacteria were found to be more positive in calculous gallbladders (86/96, 89.6%) than in acalculous gallbladders (2/4, 50%).

Table 5: Comparison between calculous and acalculous cases.

Parameter	Calculous (n=96)	Acalculous (n=4)	P-value
Histopathology positive	94 (97.9%)	1 (25%)	<0.001
Culture positive	86 (89.6%)	2 (50%)	0.041

DISCUSSION

The present study offers integrated microbiological and histopathologic data in favor of a strong correlation of bacterial colonization with gallbladder disease in the patients with cholelithiasis.[22] The results showed a high rate of positive bile culture (88%), and have revealed a high prevalence of Rokitansky–Aschoff sinuses, especially, in addition to other chronic histopathological changes, with Escherichia coli being the most frequently isolated organisms. The results confirm the new idea that cholelithiasis is not just a metabolic disorder but that there is an important infectious and inflammatory component to it.[23,24]

The high percentage of isolation of bacteria in this study aligns with recent studies carried out in the last six years (2020-2026), which have challenged the classic notion that bile is sterile in normal circumstances.[25] Recent molecular and culture-based research has shown that gallbladder bile from non-infected patients can contain bacterial DNA and viable organisms, indicating that the presence of bacteria in the gallbladder bile may be more central to gallstone disease than they are currently believed to be.[26] E. coli was the most prevalent pathogen in the current cohort, consistent with its known tendency to colonise the biliary tract, form biofilms and survive in bile-rich environments, which may help to nucleate and persist stones.[27]

The link between bacterial infection and gallstone disease observed could arise from several mechanisms. It has been suggested that a route to pigment stone formation may be through bacterial enzymes, such as the β-glucuronidase enzyme, that can deconjugate bilirubin. Furthermore, bacterial biofilms can serve as morphological structures, which can promote cholesterol crystal aggregation and gallstone formation. More recent experimental research (2021–2025) has also indicated that bacterial lipopolysaccharides may result in mucin overproduction, which leads to cirrhosis and bile hyperviscosity and thus to lithogenesis. All of these mechanisms help to maintain a multifactorial model, in which infection synergizes with metabolic and biochemical factors.[28,31]

Gallstone disease is a chronic inflammatory condition and the histopathological findings in this study further support the chronic inflammatory nature of the disease.[32] Rokitansky–Aschoff sinuses, chronic inflammatory infiltration, mucosal hyperplasia and fibrosis indicate long-standing gallbladder injury. These lesions have also been found in recent, large-scale histopathological studies and have always been linked to chronic gallstone status and recurrent inflammation. In recent literature, Rokitansky–Aschoff sinuses, especially, have increasingly been identified as potential microenvironments which are conducive to bacterial persistence, bile stasis and ongoing mucosal damage.[33]

Importantly, the strong correlation between positive bacterial culture and advanced histopathological changes noted in the present study may reflect a causal relationship, although it could be coincidental. There was a significant increase in odds for structural gallbladder damage in patients with bacterial colonization, which supports the hypothesis that infection may promote chronic inflammatory remodeling.[34] The results of this study are in line with recent cohort studies that have found analogous associations between the composition of the biliary microbiota and the severity of gallbladder inflammation.[35]

The overall culture positivity rate in the present study (88%) is relatively higher compared with some of the western countries but of similar proportions when compared to literature found in South Asian and the Middle East countries. These differences can be explained by the variations in environmental factors, dietary intakes, health care access, and surgical intervention. Geographic variability in the composition of biliary microbiota has recently been highlighted and could affect disease progression and treatment response.[36,37]

In one fourth of positive cases, growth of more than one microorganism was observed, further emphasizing the complexity of the biliary microbial ecosystems. More recent microbiome-based research has shown that most gallbladder infections are not monocausal, but are composed of multiple bacterial communities that can interact synergically.[38] This may account for why some patients continue to have inflammation and why gallstone disease tends to recur in some patients.[39]

The results of this study have clinical implications. Because of the high prevalence of bacterial colonization, the potential for directed antimicrobial therapy in selected patients, especially those with complicated or recurrent disease, should be considered. However, recently published guidelines on the clinical use of antibiotics in cholelithiasis remain wary of the routine use of antibiotics for cholelithiasis, and they state that additional randomized controlled trials are required to establish the therapeutic benefit of antibiotic use. Furthermore, the presence of certain patterns of bacteria in this population could help to formulate region-specific management guidelines.[40,41]

There are a number of limitations to be recognized in spite of these strengths. A cross-sectional study is not able to draw firm causal inferences linking bacterial infection with the development of gallstones. Moreover, traditional culture-based methods could underestimate the actual diversity of biliary microbiota relative to recent molecular methods like 16S rRNA sequencing. Further research with metagenomic techniques and long-term follow-up is warranted to further determine the temporal association between infection and gallstone pathogenesis.[42,43]

Finally, in this study, there is good evidence linking the presence of bacterial colonization, especially Escherichia coli, to the histopathological changes of cholelithiasis.[44] The results are part of a growing body of evidence that suggests that gallstone disease is a multifactorial event involving metabolic, inflammatory and infectious pathways.[45,46] The understanding of this interaction may lead to new possibilities for prevention and specific treatment.[47]

CONCLUSION

In this study, there was a strong correlation between bacterial colonization and histopathological changes in those having cholelithiasis. The above high positive bile culture rate, mainly of Escherichia coli, confirms the hypothesis that microbial involvement is not just coincidental, but could be a contributing factor to the genesis of gallstones. The high rate of chronic inflammatory changes such as Rokitansky–Aschoff sinuses, mucosal hyperplasia and fibrosis further underscores the chronic inflammatory nature of gallbladder disease and the potential role of persistent bacterial presence.

The correlation between the culture positivity and the higher stages of histopathological damage indicated that bacterial infection could be a worsening factor in the process of gallbladder damage and progression. All of these results together support the idea that cholelithiasis is a multifactorial disease that includes metabolic, inflammatory and infectious mechanisms and is not solely a biochemical disorder.

Recommendations

1. Future studies should integrate cutting-edge molecular diagnostic methods, such as 16S rRNA sequencing and metagenomic analysis, to better define the biliary microbiome and to work around the shortcomings of traditional culture approaches.
2. Longitudinal and multicenter studies are suggested to further clarify temporal and causal links between bacterial colonization and the development of gallstones.
3. Antimicrobial susceptibility testing of the isolated biliary pathogens should be performed in complicated cases for evidence-based empirical antibiotic therapy.
4. Bacterial eradication may be included as a therapeutic approach to cholelithiasis in selected patients during clinical trials to assess its role in influencing disease progression/reoccurrence and/or in the prevention of cholelithiasis.
5. Regional surveillance studies should be done in order to detect geographic variations in biliary microbiota and resistance patterns especially in developing health care settings.
6. Incorporation of microbiological data into metabolic and genetic risk analysis could lead to a better understanding of the pathogenesis of gallstone disease and assist in personalized treatment.

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