

Assessment of Fecal *Lactobacillus* spp Distribution as a Biomarker for Dysbiosis in Iraqi Post-Cholecystectomy Patients

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ABSTRACT

Cholecystectomy is a highly prevalent procedure in Iraq for treating cholelithiasis; nonetheless, many patients develop post-cholecystectomy syndrome (PCS). Emerging molecular studies suggest that 'biliary dysbiosis,' caused by the constant input of bile acids into the intestinal lumen, is a major cause of these symptoms. The purpose of this study was to determine the distribution of *Lactobacillus* spp. as a potential biomarker for dysbiosis, as well as its relationship to gastrointestinal distress in Iraqis. A case-control clinical investigation was carried out at Baghdad Hospital for Digestive Diseases with 100 participants (40 males and 60 females) aged 40 to 60 years. The participants were separated into two groups: a control group of 50 patients with symptomatic cholelithiasis (pre-operative) and a case group of 50 patients who had a cholecystectomy 12 weeks before. Fecal samples were tested using *16S rRNA* gene-specific PCR to assess the prevalence and relative band intensity of *Lactobacillus* species. The microbial distribution was evaluated statistically, using chi-square and densitometric methods, in connection to clinical complaints. The data showed that the prevalence and relative density of *Lactobacillus* species were significantly lower in the post-cholecystectomy group than in the control group. These microbial changes had a strong negative connection ($r = -0.78$) with the intensity of gastrointestinal distress. The loss of gallbladder function caused a dramatic "microbial shift" in the intestinal environment, which impacted the stability of helpful lactic acid bacteria. The findings demonstrate that gallbladder resection creates a distinct microbial imbalance in the Iraqi population. In Iraqi clinics, restoring microbial balance with tailored probiotic drugs and dietary adjustments shows promise as a therapeutic technique for managing PCS symptoms.

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1- INTRODUCTION

The human gut microbiota is a complex microbial ecosystem that maintains host metabolic and immunological equilibrium. The enterohepatic circulation of bile acids is an important regulator of this ecosystem, with strong antibacterial properties [1] and a direct influence on the microbial makeup of the intestines. The gallbladder serves as a vital reservoir, allowing a regulated, postprandial flow of concentrated bile into the duodenum in response to dietary fat intake. Cholecystectomy, or surgical removal of the gallbladder, disrupts this

physiological synchronization, resulting in an uncontrolled flood of bile acids into the small intestine [2]. This physiological transition is typically accompanied by "biliary dysbiosis," which is defined by major changes in the composition and stability of the gut microbiota [3]. Previous research has produced conflicting results regarding these microbial shifts; while some studies indicate a transient change that stabilizes within months [4], others suggest long-term alterations in specific taxa, such as a decrease in Bacteroidetes and an increase in Firmicutes. These microbial imbalances are often linked to post-cholecystectomy syndrome (PCS), which is characterized by persistent diarrhea, bloating, and abdominal pain. In many clinical cases, these microbial imbalances are linked to post-cholecystectomy syndrome (PCS), which is characterized by persistent diarrhea, bloating, and stomach pain [5].

In Iraq, cholecystectomy is one of the most common abdominal procedures [6]. Despite its popularity, there has been a considerable lack of molecular-based research into the impact of this surgery on the gut flora, particularly among Iraqi groups. Given Iraq's unique dietary patterns, which include high fat and caloric intake, the microbial response to unregulated bile flow may differ greatly from Western or East Asian populations [7].

The Firmicutes/Bacteroidetes (F/B) ratio is an important measure of intestinal balance, gut health, and metabolic stability [5]. Under normal physiological conditions, a balanced F/B ratio preserves the intestinal barrier and controls systemic inflammation. However, changing bile acid dynamics after cholecystectomy can significantly upset this ratio [2]. *Lactobacillus*, a member of the Firmicutes phylum, is an important biomarker in this setting. Assessing the distribution and relative density of these species provides molecular insight into the microbiological influence of gallbladder resection and subsequent PCS formation [3].

The aim of the study can be summarized as follows: To determine the distribution and relative density of fecal *Lactobacillus* spp. as a potential biomarker for dysbiosis in Iraqi post-cholecystectomy patients. To evaluate the relationship between these microbial changes and the severity of gastrointestinal symptoms associated with post-cholecystectomy syndrome (PCS). To assess how gallbladder resection alters the intestinal microbial environment in the Iraqi population, considering local dietary patterns.

2- MATERIALS AND METHODS

2.1 Research Design and Participant Enrollment

This case-control clinical investigation aimed to determine the microbiological distribution of gut *Lactobacillus* spp. in relation to elective laparoscopic cholecystectomy. The study was carried out in the Baghdad Hospital for Digestive Diseases (Medical City, Iraq), with one hundred (100) participants. The Institutional Review Board (IRB) granted ethical approval, and all participants gave written informed consent. To assure clinical accuracy, data was collected from October 10, 2025 to January 30, 2026. Participants were placed into two different groups.

2.2 Sample Collection and Preservation

Participants from both groups provided fecal samples. Samples for the Case Group were collected after a 12-week post-operative follow-up to allow the biliary-intestinal environment to stabilize [4]. The samples were gathered in sterile, DNA-free containers. To retain microbiological integrity and prevent DNA degradation, about 200 mg aliquots were placed into sterile 1.5 ml Eppendorf tubes and promptly stored at -80°C even make molecular analysis [8].

2.3 DNA Extraction and Quality Control

Total microbial genomic DNA was extracted from 200 mg of fecal matter using the QIAamp Fast DNA Stool Mini Kit (QIAGEN, Germany), closely adhering to the manufacturer's pathogen-rich sample guidelines. The extracted DNA's concentration and purity (A260/A280 ratio) were measured using a NanoDrop™ spectrophotometer to achieve a minimum concentration of (50 ng/μL) for PCR amplification [8].

2.4 Target-Specific Primers and Bioinformatics Verification

To address the study's focus on critical microbial markers, 16S rRNA gene-specific primers were used to detect and quantify *Lactobacillus* spp. and other relevant taxa [9]. The sequences were thoroughly cross-referenced with the Ribosomal Database Project (RDP) and NCBI GenBank to ensure excellent taxonomic specificity within the Iraqi microbial context [10].

Target-specific primers for *Lactobacillus* spp. were designed with forward sequence 5'-CACGCTGCACAGAGAGCTTT-3' and reverse sequence 5'-TGCAACTTTCCTCTTGCTGC-3'. These primers were synthesized by Macrogen (Seoul, South Korea) and validated to avoid non-specific amplification of coexisting gut microflora. PCR amplification was performed using a Bio-Rad T100™ Thermal Cycler. The 25 µL reaction mixture consisted of 12.5 µL of 2x HotStarTaq Master Mix (QIAGEN), 1 µL of each primer (10 pmol), 2 µL of template DNA, and 8.5 µL of nuclease-free water.

The optimal thermal profile included an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 45 seconds, with a final extension at 72°C for 10 minutes [4]. The integrity and specificity of the PCR products were verified via 2% agarose gel electrophoresis at 80V for 45 minutes [11]. Visualization under UV light ensured the presence of distinct bands at the expected molecular weights without non-specific artifacts [12].

2.6 Densitometry Analysis and Semi-Quantification

To account for the qualitative nature of traditional PCR, semi-quantitative analysis was used. A Gel Documentation System was used to record digital photographs of the 2% agarose gels. The relative strength and density of the particular 16S rRNA bands for *Lactobacillus* spp. were measured with ImageJ software (NIH, USA) [13]. The integrated density of each band was standardized against a molecular weight marker to calculate the relative microbial load in the control and case groups [12].

2.7 Statistical Analysis and Data Interpretation

The data were analyzed with SPSS Version 26.0 (Field, 2024). The Chi-square test was used to compare the prevalence of microbial targets in the two groups. The mean band intensities between the control and case groups were compared using the Independent Samples T-test using the semi-quantitative densitometry data. Pearson's correlation coefficient (r) was used to calculate the relationships between microbial changes and clinical symptom severity. Statistical significance was defined as a p-value < 0.05.

3- RESULTS AND DISCUSSION

3.1 Targeted Microbial Prevalence and Density Analysis

The molecular study uses *16S rRNA* gene-specific PCR revealed considerable changes in gut microbial structure after cholecystectomy. According to the "biliary dysbiosis" concept, the constant input of bile acids into the intestinal lumen preferentially reduced sensitive commensal taxa. *Lactobacillus* spp. detection frequency and intensity decreased significantly in the post-operative group (Case) compared to the symptomatic gallstone group (Control) ($p < 0.05$).

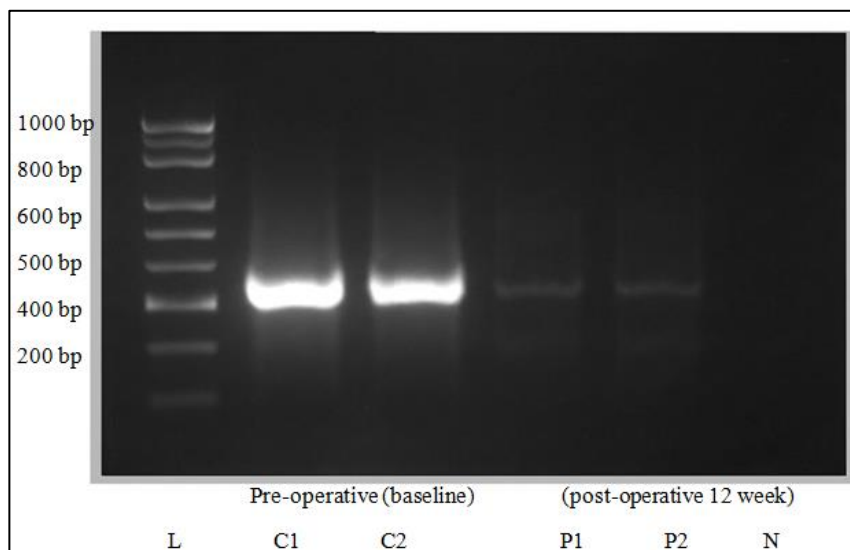


Fig (1): Agarose Gel Electrophoresis Analysis.

Agarose gel electrophoresis (2%) showing quantitative PCR amplification of *Lactobacillus spp. 16S rRNA* gene (Product Size: 340 bp). Lanes L: 100 bp DNA Ladder. , Lanes C1, C2: Representative samples from the Control group showing high band intensity. Lanes P1, P2: Representative samples from the Case group (12 weeks' post-op) showing significantly reduced (faint) band intensity. Lane N: Negative Control.

This considerable drop shows that the gallbladder is an important regulator of intestinal homeostasis [14]. Following surgery, the persistent "trickling" of bile acids has a detergent-like effect on the gut lining [15]. Our findings indicate that this changed habitat is detrimental to commensal bacteria that are sensitive to bile acid toxicity [3].

3.2 Semi-Quantitative Analysis of Lactobacillus spp.

The relative microbial load was estimated by quantifying the integrated density of the PCR bands using ImageJ software. Table (1) shows a considerable drop in *Lactobacillus ssp* levels.

Table (1): Comparison of Lactobacillus spp relative band intensity and prevalence between groups

Group	Prevalence (%)	Mean Relative Band Intensity (Pixels)	P-Value
Control Group (n=50)	88.0%	1450 \pm 120	(Ref)
Case Group (n=50)	62.0%	480 \pm 85	< 0.001

The significant drop in density ($p < 0.01$) indicates a functional loss. Gram-positive bacteria such as *Lactobacillus* are extremely susceptible to primary bile acids, which serve as surfactants and damage bacterial cell membranes [14]. This depletion, which may be aggravated by the Iraqi diet's high fat content, allows for a microbial shift.

3.3 Prevalence of Gastrointestinal Symptoms (PCS)

Post-operative symptoms were evaluated to determine the clinical manifestations of Post-Cholecystectomy Syndrome (PCS) in the research population.

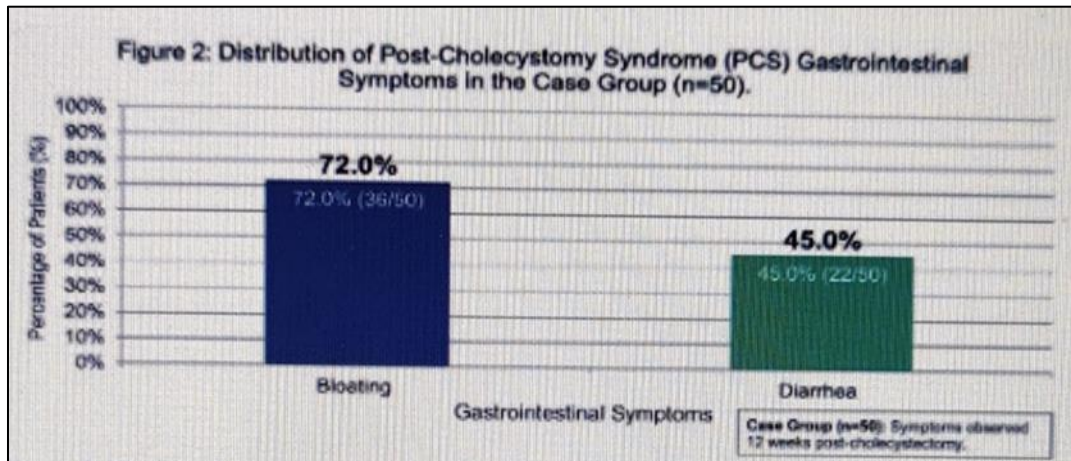


Fig (2): Distribution of gastrointestinal symptoms in the Case Group (n=50). The chart highlights the dominance of bloating (72.0%) and diarrhea (45.0%) as primary manifestations of PCS.

The 72% incidence of bloating is directly related to *Lactobacillus* deficiency. The lowering of these bacteria reduces fermentation efficiency, creating an environment that promotes gas-producing colonies [12]. Diarrhea (45%) is caused by the osmotic effects of uncontrolled bile flow, indicating a microbiological and osmotic basis for PCS [16].

3.4 Correlation between Microbial Density and Clinical Symptoms

A strong statistical correlation was identified between the reduction in *Lactobacillus* density and the severity of clinical symptoms.

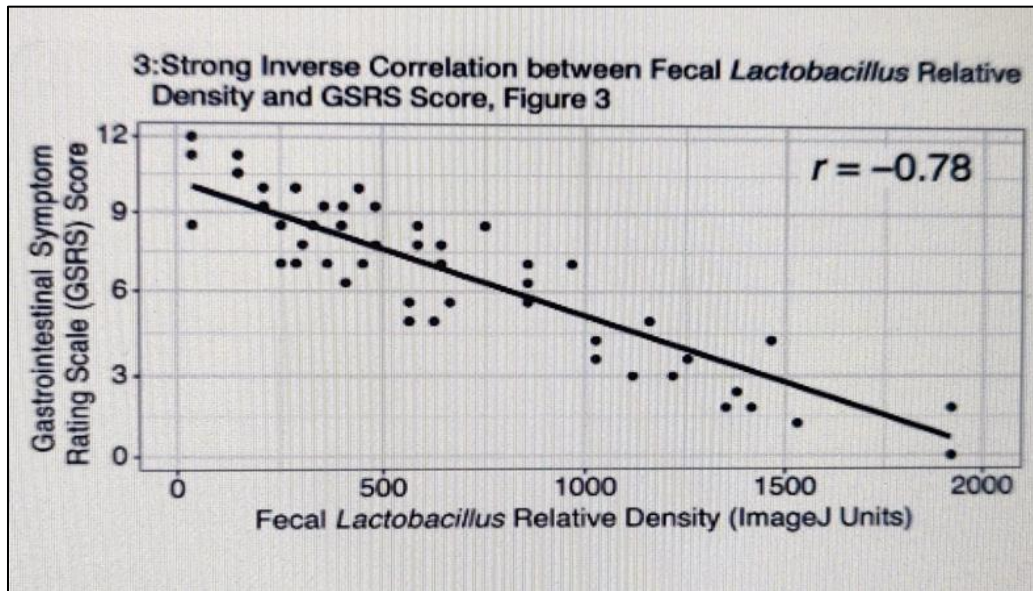


Fig (3): Shows a scatter plot of the strong inverse connection ($r = -0.78$) between fecal *Lactobacillus* relative density (ImageJ units) and GSRS score

The significant inverse association ($r = -0.78$) indicates a solid biological basis for PCS. Patients with the lowest densitometric values reported the most severe bloating and diarrhea ($p < 0.01$). This demonstrates that the absence of helpful bacteria is a crucial predictor of intestinal distress in Iraqi patients [14].

4- CONCLUSION

4.1 Conclusion

In conclusion, this study indicates that surgical removal of the gallbladder causes a significant "microbial and chemical shift" in the intestinal environment. Our molecular results, confirmed by PCR and densitometry analysis, show that cholecystectomy causes considerable modifications in gut microbial ecology. This dysbiosis is characterized by a significant decrease in the prevalence and relative density of beneficial taxa, particularly *Lactobacillus* spp. The persistent, unregulated flow of bile acids into the colon during surgery serves as a selective biological stressor, disrupting microbial balance. This imbalance is closely linked to the start of Post-Cholecystectomy Syndrome (PCS) symptoms, such as bloating and gastrointestinal distress, as demonstrated by the considerable inverse correlation. As a result, these symptoms should be treated therapeutically as a chronic microbiological and osmotic imbalance rather than a temporary stage of surgical recovery. *Lactobacillus* spp. is a reliable molecular biomarker for determining the degree of this dysbiosis in the Iraqi clinical setting.

4.2 Clinical Implication

Clinical recommendations for managing post-cholecystectomy syndrome (PCS) include the use of targeted probiotic medicines, such as *Lactobacillus* strains, to help restore microbial balance after surgery; additionally, Iraqi patients should be advised to consume a low-fat, fiber-rich diet following cholecystectomy to reduce the detergent-like effects of constant bile flow. Molecular monitoring via *16S rRNA* screening is useful for diagnosing chronic PCS symptoms and assessing microbial depletion, while future research plans in Iraq aim to conduct larger multi-center investigations using Real-Time PCR (qPCR) or Next-Generation Sequencing (NGS) to map the post-cholecystectomy microbiota more comprehensively.

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