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Research Article

STUDY OF BACTERIAL FLORA OF GALL BLADDER IN DIFFERENT SUBSETS OF PATIENTS OF CHOLELITHIASIS

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ABSTRACT

Background: Gallstone disease is frequently associated with bacterial colonisation of bile, particularly in patients with cholecystitis and those undergoing endoscopic retrograde cholangiopancreatography (ERCP). The presence of bacteria in bile may influence disease severity, choice of antibiotics, and intraoperative difficulty during cholecystectomy. However, the sterility of bile in asymptomatic gallstones and the impact of ERCP on biliary contamination remain areas of clinical uncertainty.

Objectives: To study the pattern of bacterial flora and antibiotic sensitivity in bile aspirated from the gallbladder among different clinical subsets of cholelithiasis, to assess bile sterility in asymptomatic gallstones, to evaluate bile contamination following ERCP and sphincterotomy as evidence of duodeno-biliary reflux, and to correlate bile culture positivity with intraoperative difficulty during cholecystectomy.

Methods: This prospective observational study was conducted over a period of three years at a tertiary care centre. Seventy-five patients undergoing laparoscopic or open cholecystectomy were included and divided equally into three groups: asymptomatic/uncomplicated gallstones (Group A), acute or chronic cholecystitis (Group B), and post-ERCP patients with choledocholithiasis (Group C). Bile was aspirated intraoperatively under sterile conditions and subjected to microbiological culture and antibiotic sensitivity testing. Clinical presentation, laboratory parameters, bile culture results, antibiotic sensitivity patterns, and intraoperative difficulty were analysed. Statistical analysis was performed using chi-square test and Student's *t*-test, with $p < 0.05$ considered statistically significant.

Results: Bile culture positivity was observed in 40% of patients overall, with a significantly higher rate in post-ERCP patients (80%) compared to cholecystitis (32%) and asymptomatic gallstones (8%). Enteric gram-negative bacilli constituted 86.7% of isolates, with *Escherichia coli* (46.7%) being the most common organism, followed by *Pseudomonas aeruginosa* (26.7%) and *Klebsiella pneumoniae* (13.3%). Antibiotic sensitivity was highest for carbapenems (93.3%), followed by amikacin (80%) and piperacillin–tazobactam (73.3%). Difficult cholecystectomy was significantly more common in patients with positive bile cultures, particularly in the post-ERCP group (84%), indicating a strong association between biliary contamination and operative difficulty.

Conclusion: Bile in asymptomatic gallstone disease is largely sterile, whereas significant bacterial contamination is seen in cholecystitis and especially following ERCP, supporting the role of duodeno-biliary reflux. Enteric gram-negative organisms predominate, and bile contamination correlates with increased intraoperative difficulty. Targeted antibiotic therapy based on local sensitivity patterns is essential, particularly in post-ERCP patients, to optimise surgical outcomes.

Keywords*: Cholelithiasis; Bile culture, ERCP; Cholecystitis, Duodeno-biliary reflux, Antibiotic sensitivity, Cholecystectomy difficulty.*

INTRODUCTION

Cholelithiasis is one of the most common biliary tract disorders encountered in surgical practice worldwide, with a reported prevalence ranging from 10–20% in the adult population in Western countries and an increasing trend in developing nations, including India [1]. The burden of gallstone disease in India has shown a steady rise over the past few decades, attributed to changing dietary habits, increasing obesity, metabolic syndrome, and improved diagnostic imaging such as ultrasonography and computed tomography [2]. Gallstones are more commonly observed in females, particularly in the reproductive age group, and the disease spectrum ranges from asymptomatic gallstones to complicated presentations such as acute cholecystitis, choledocholithiasis, gallstone pancreatitis, and biliary sepsis [3].

Traditionally, bile within the gallbladder has been considered sterile; however, accumulating evidence suggests that bacterial colonization of bile plays a significant role in the pathogenesis and progression of gallstone disease and its complications [4]. Several studies have demonstrated that bacteria can be isolated from bile in patients with acute and chronic cholecystitis, with reported culture positivity rates ranging from 20% to 60%, depending on the clinical presentation and prior interventions [5]. The presence of bacteria in bile has been implicated in gallstone formation, inflammation of the gallbladder wall, postoperative infective complications, and increased difficulty during cholecystectomy [6].

The spectrum of bacterial flora isolated from bile predominantly consists of enteric organisms, particularly Gram-negative bacilli such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, suggesting an ascending route of infection from the duodenum into the biliary system [7]. This ascending infection is believed to occur due to duodeno-biliary reflux, especially in the presence of sphincter of Oddi dysfunction or after endoscopic interventions [8]. Gram-positive organisms and anaerobes have also been reported, albeit less frequently, particularly in complicated biliary infections [9].

Endoscopic Retrograde Cholangiopancreatography (ERCP) has become an essential therapeutic modality for the management of choledocholithiasis, biliary obstruction, and gallstone pancreatitis. While ERCP significantly reduces morbidity and mortality associated with biliary obstruction, it is also known to disrupt the natural barrier function of the sphincter of Oddi, especially following sphincterotomy [10]. This disruption facilitates duodeno-biliary reflux, leading to contamination of bile with enteric organisms and increasing the risk of ascending cholangitis and gallbladder infection [11]. Studies have reported significantly higher bile culture positivity rates in patients who have undergone ERCP compared to those without prior endoscopic intervention [12].

The presence of bacterial contamination in bile has important clinical implications. It has been associated with increased operative difficulty during cholecystectomy due to dense adhesions, inflamed Calot's triangle, friable gallbladder wall, and higher conversion rates from laparoscopic to open surgery [13]. Moreover, inappropriate empirical antibiotic use without knowledge of local bacterial flora and sensitivity patterns may contribute to postoperative infections, antimicrobial resistance, and prolonged hospital stay [14].

Despite the clinical importance of bile microbiology, there remains a lack of consensus regarding the sterility of bile in asymptomatic gallstone disease, the impact of ERCP-related contamination, and the optimal choice of perioperative antibiotics based on regional sensitivity patterns [15]. Most available studies are heterogeneous, limited by small sample sizes, or focus on a single clinical subset of gallstone disease [16]. There is also limited Indian data comparing bile culture findings across different clinical presentations of cholelithiasis, particularly in patients with prior ERCP intervention [17].

In this context, the present prospective study was undertaken to evaluate the bacterial flora of gallbladder bile in patients with three distinct clinical subsets of cholelithiasis—patients with asymptomatic or uncomplicated gallstones, patients with acute or chronic cholecystitis, and patients with choledocholithiasis who had undergone ERCP. The study further aims to analyze antibiotic sensitivity patterns, assess evidence of duodeno-biliary reflux, and correlate bile culture findings with intraoperative difficulty during cholecystectomy, thereby providing clinically relevant evidence to guide perioperative management and antibiotic selection in gallstone disease.

The present prospective study was undertaken to evaluate the pattern of bacterial flora and their antibiotic sensitivity in bile aspirated from the gallbladder in patients with cholelithiasis presenting in three distinct clinical subsets—patients with asymptomatic or uncomplicated gallstones, patients with acute or chronic cholecystitis, and patients with choledocholithiasis who had undergone ERCP interventions. The study further aimed to assess whether bile in asymptomatic gallstone disease is truly sterile, to determine the extent of bile contamination following ERCP and sphincterotomy, and to evaluate the presence of duodeno-biliary reflux through the frequent isolation of enteric organisms. An additional objective was to correlate bile culture findings with intraoperative difficulty during cholecystectomy and to identify the most appropriate antibiotic regimen based on local sensitivity patterns. The justification for this study lies in the rising incidence of gallstone disease, the increasing use of ERCP, the lack of consensus regarding bile sterility in different clinical scenarios, and the growing concern of antimicrobial resistance. By generating institution-specific microbiological data, this study seeks to provide evidence-based guidance for perioperative antibiotic selection and surgical planning, thereby improving patient outcomes and reducing postoperative morbidity in gallstone disease.

METHODOLOGY

This prospective observational study was conducted at a tertiary health care centre and medical college located in a metropolitan city over a duration of three years, following approval from the Institutional Ethics Committee. The study population comprised patients diagnosed with gallstone disease who were admitted for elective or emergency cholecystectomy during the study period. A total sample size of 75 patients was included using a prospective sampling approach. Written informed consent was obtained from all participants prior to enrolment. Patients were categorized into three equal groups of 25 each based on their clinical presentation and prior biliary interventions. Group A included patients with asymptomatic or uncomplicated gallstones detected incidentally on ultrasonography or computed tomography, without any history or clinical, laboratory, or radiological evidence suggestive of acute or chronic cholecystitis. Group B consisted of patients diagnosed with acute or chronic cholecystitis based on clinical features such as right hypochondrial pain, fever, positive Murphy's sign, leukocytosis (WBC >11,000/mm^3), and radiological findings including gallbladder wall thickening greater than 3 mm, pericholecystic fluid, fat stranding, or sonographic Murphy's sign. Group C comprised patients with choledocholithiasis, gallstone pancreatitis, or gallstone-related obstructive jaundice who had previously undergone ERCP with sphincterotomy, with or without stone extraction or biliary stenting.

Patients undergoing cholecystectomy for indications other than gallstone disease, such as gallbladder carcinoma, perforation, empyema, mucocoele, gallbladder without stones, or cholecystectomy performed as part of another major abdominal procedure, were excluded from the study. Additionally, patients in Group A who later demonstrated features of cholecystitis and patients in Group B lacking inflammatory features were excluded to maintain strict group delineation. For Group C, patients who had undergone ERCP for non-gallstone-related biliary conditions were also excluded. A detailed clinical history was obtained for all patients, including symptoms of biliary colic, fever, jaundice, and prior hospitalizations. Relevant laboratory investigations such as total leukocyte count and liver function tests were recorded, and radiological findings were reviewed to confirm group allocation.

During laparoscopic or open cholecystectomy, bile was aspirated directly from the gallbladder using a sterile 22-gauge angiocath attached to a 10 mL syringe, prior to gallbladder manipulation and after achieving adequate exposure. Strict aseptic precautions were maintained during sample collection. The aspirated bile sample was immediately transferred in sterile containers to the microbiology laboratory of the institute for aerobic culture and antibiotic sensitivity testing using standard culture techniques. Wherever feasible, preoperative antibiotics were withheld to avoid interference with culture results, although patients may have received antibiotics during prior episodes of cholecystitis or biliary intervention. Culture and sensitivity results were typically available within four days. The isolated organisms were identified, and antibiotic susceptibility patterns were documented for each positive culture.

Intraoperative findings were systematically recorded, including the presence of adhesions, gallbladder wall thickness, difficulty in dissection at Calot's triangle, need for conversion to open surgery, and overall difficulty level of cholecystectomy. These findings were correlated with bile culture results to assess the association between bacterial colonization and surgical difficulty. Data were entered into a structured database and analyzed using standard statistical methods. Categorical variables were analyzed using Chi-square tests and continuous variables using Student's t-test, with a p-value of less than 0.05 considered statistically significant.

RESULTS

A total of 75 patients with gallstone disease undergoing cholecystectomy were included in this prospective study, of whom 58 (77.3%) were females and 17 (22.7%) were males, showing a clear female preponderance. The majority of patients belonged to the 31–50 years age group (47 patients; 62.7%), followed by those aged more than 50 years (17 patients; 22.7%) and up to 30 years (11 patients; 14.7%). The study population was equally divided into three clinical subsets, with 25 patients (33.3%) each in Group A (asymptomatic/uncomplicated gallstones), Group B (acute/chronic cholecystitis), and Group C (post-ERCP patients).

Clinically, right hypochondrial pain was the most common presenting symptom, reported in 69 patients (92.0%), with universal presence in Groups B and C. Fever was documented in 39 patients (52.0%), predominantly among patients with cholecystitis (80.0% in Group B) and post-ERCP patients (68.0% in Group C), highlighting its strong association with inflammatory gallbladder pathology. History suggestive of obstructive jaundice was observed in 21 patients (28.0%), the majority of whom belonged to the post-ERCP group (76.0%), reflecting the biliary obstruction profile of this subset.

Bile culture analysis revealed bacterial growth in 30 patients (40.0%), while 45 patients (60.0%) had sterile bile. Culture positivity was markedly higher in post-ERCP patients (80.0%), followed by patients with acute/chronic cholecystitis (32.0%), whereas bile from asymptomatic gallstone patients was largely sterile, with only 8.0% showing bacterial growth. Among culture-positive samples, enteric gram-negative bacilli constituted the predominant organisms (86.7%), supporting the possibility of ascending duodeno-biliary reflux, particularly in post-sphincterotomy patients. *Escherichia coli* was the most frequently isolated organism (46.7%), followed by *Pseudomonas aeruginosa* (26.7%) and *Klebsiella pneumoniae* (13.3%).

Antibiotic sensitivity testing demonstrated high susceptibility to carbapenems (93.3%), followed by amikacin (80.0%) and piperacillin–tazobactam (73.3%), while sensitivity to third-generation cephalosporins (40.0%) and fluoroquinolones

(30.0%) was comparatively lower. These findings suggest the need for broader-spectrum antibiotic coverage, particularly in post-ERCP patients and those with cholecystitis.

Intraoperatively, difficult cholecystectomy was encountered in 40 patients (53.3%), with the highest difficulty rate observed in post-ERCP patients (84.0%), followed by those with acute/chronic cholecystitis (52.0%). Asymptomatic gallstone patients had the lowest difficulty rate (24.0%). This association between clinical subset and operative difficulty was statistically significant ($\chi^2 = 18.11$, $p = 0.0001$), indicating that prior ERCP and bile contamination substantially increase surgical complexity.

Overall, the study demonstrates a strong association between clinical presentation, bile contamination, bacterial flora, antibiotic sensitivity patterns, and intraoperative difficulty of cholecystectomy, with post-ERCP patients representing the highest-risk subgroup.

Table 1. Demographic Profile and Clinical Subsets of Study Participants (n = 75)

Variable	Category	Frequency (n)	Percentage (%)
Sex	Male	17	22.7
	Female	58	77.3
Age group (years)	≤ 30	11	14.7
	31–50	47	62.7
	> 50	17	22.7
Clinical subset (study group)	Group A – Asymptomatic / uncomplicated gallstones	25	33.3
	Group B – Acute / chronic cholecystitis	25	33.3
	Group C – Post-ERCP patients	25	33.3
Total		**75**	**100.0**

Table 2. Clinical Presentation and Bile Culture Findings Across Study Groups (n = 75)

Parameter	Category	Group A – Asymptomatic / Uncomplicated Gallstones (n = 25)	Group B – Acute / Chronic Cholecystitis (n = 25)	Group C – Post-ERCP Patients (n = 25)	Total (n = 75)
Right hypochondrial pain	Present	19 (76.0%)	25 (100.0%)	25 (100.0%)	69 (92.0%)
	Absent	6 (24.0%)	0 (0.0%)	0 (0.0%)	6 (8.0%)
History of fever	Present	2 (8.0%)	20 (80.0%)	17 (68.0%)	39 (52.0%)
	Absent	23 (92.0%)	5 (20.0%)	8 (32.0%)	36 (48.0%)
History suggestive of obstructive jaundice	Present	0 (0.0%)	2 (8.0%)	19 (76.0%)	21 (28.0%)
	Absent	25 (100.0%)	23 (92.0%)	6 (24.0%)	54 (72.0%)
Bile culture result	Culture positive	2 (8.0%)	8 (32.0%)	20 (80.0%)	30 (40.0%)
	Culture sterile	23 (92.0%)	17 (68.0%)	5 (20.0%)	45 (60.0%)
Type of organisms isolated*	Enteric gram-negative bacilli	1 (50.0%)	5 (62.5%)	20 (100.0%)	26 (86.7%)
	Non-enteric organisms	1 (50.0%)	3 (37.5%)	0 (0.0%)	4 (13.3%)

* Percentages for type of organisms are calculated among **culture-positive samples only**.

Table 3. Spectrum of Bacterial Isolates and Antibiotic Sensitivity Pattern in Bile Samples (n = 30)

Parameter	Category	Frequency (n)	Percentage (%)
Bacterial isolates identified	*Escherichia coli*	14	46.7
	Pseudomonas aeruginosa	8	26.7
	Klebsiella pneumoniae	4	13.3
	Enterococcus species	2	6.7
	Mixed / other organisms	2	6.7
	Total isolates	**30**	**100.0**
Antibiotic sensitivity pattern	Carbapenems	28	93.3
	Amikacin	24	80.0
	Piperacillin + Tazobactam	22	73.3
	Third-generation cephalosporins	12	40.0
	Fluoroquinolones	9	30.0

Note: Antibiotic sensitivity percentages are calculated **among culture-positive isolates (n = 30)**. Multiple isolates per patient were possible.

Table 4. Intraoperative Difficulty of Cholecystectomy in Relation to Bile Culture Status (n = 75)

Study Group	Difficult Cholecystectomy n (%)	Easy Cholecystectomy n (%)	Chi-square (χ^2)	p-value
Group A – Asymptomatic / Uncomplicated Gallstones (n = 25)	6 (24.0%)	19 (76.0%)		
Group B – Acute / Chronic Cholecystitis (n = 25)	13 (52.0%)	12 (48.0%)		
Group C – Post-ERCP Patients (n = 25)	21 (84.0%)	4 (16.0%)	**18.11**	**0.0001***
Total (n = 75)	**40 (53.3%)**	**35 (46.7%)**		

*** Statistically significant ($p < 0.05$)**

A statistically significant association was observed between the study groups and intraoperative difficulty of cholecystectomy ($\chi^2 = 18.11$, $p = 0.0001$). Difficult cholecystectomy was most frequently encountered in post-ERCP patients (84.0%), followed by patients with acute/chronic cholecystitis (52.0%), while the lowest difficulty rate was noted in asymptomatic gallstone patients (24.0%).

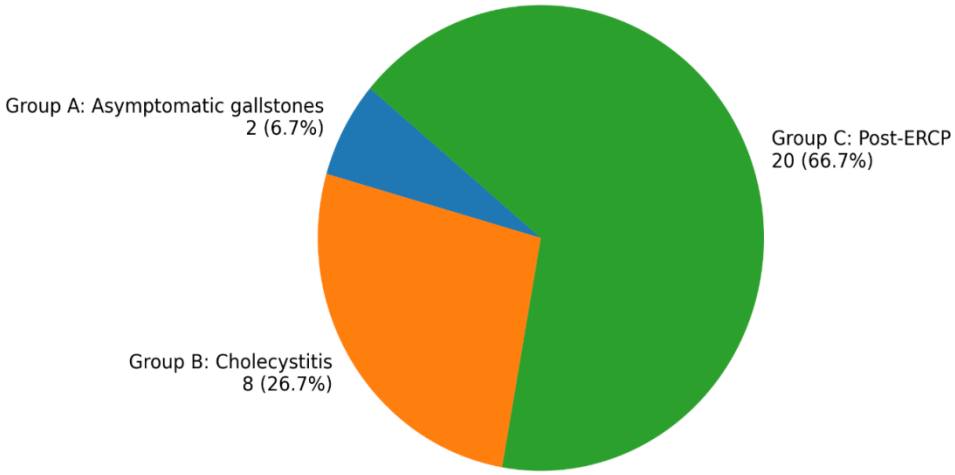

Figure 1: Distribution of Bile Culture- Positive Cases by Clinical Subset (n= 30)

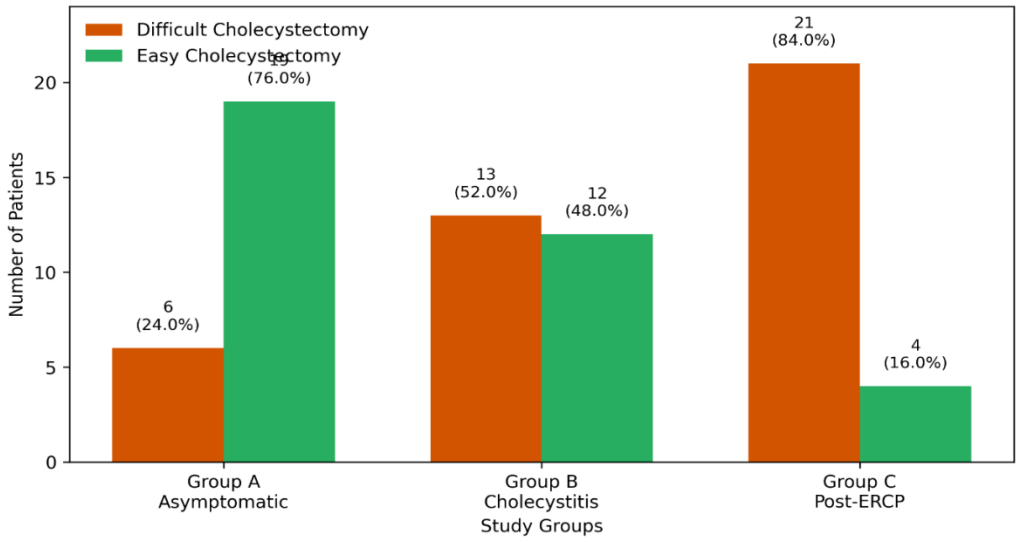

Figure 2: Intraoperative Difficulty of Cholecystectomy Across Study Groups

DISCUSSION

Gallstone disease is increasingly recognised as a condition where bacterial colonisation of bile plays a crucial role in disease progression, perioperative morbidity, and postoperative outcomes. Earlier surgical studies have highlighted that patients undergoing cholecystectomy after prior biliary interventions experience greater operative difficulty and higher inflammatory burden compared to primary uncomplicated gallstone surgery [18]. This concept forms the foundation for understanding the microbiological differences observed across the clinical subsets in the present study.

In the present study, bile culture positivity was observed in **40.0% (30/75)** of patients overall. When stratified by clinical presentation, culture positivity was lowest in **Group A (8.0%)**, intermediate in **Group B (32.0%)**, and highest in **Group C (80.0%)**. Reinders et al. [18] and Hu et al. [19] demonstrated that prior ERCP and biliary manipulation significantly increase inflammation and operative complexity, indirectly suggesting a higher likelihood of biliary contamination. Subsequent microbiological studies have corroborated these findings, reporting bile culture positivity rates ranging from **30–60%** in acute cholecystitis and exceeding **70%** in post-ERCP patients, closely mirroring the trend observed in this study [20,21].

The sterility of bile in uncomplicated gallstone disease has been debated in literature. In the present study, **92.0%** of patients with asymptomatic or uncomplicated gallstones had sterile bile. Similar findings were reported by Sunkaria et al. [22], who noted sterile bile in the majority of patients without inflammatory features, supporting the concept that bile remains sterile until obstruction, infection, or instrumentation occurs. This reinforces the hypothesis that routine prophylactic antibiotics may not be necessary in uncomplicated gallstone disease.

Analysis of bacterial flora revealed a marked predominance of **enteric gram-negative bacilli (86.7%)**, with **100% enteric organisms** isolated from post-ERCP patients. *Escherichia coli* was the most common organism (**46.7%**),

followed by *Pseudomonas aeruginosa* (**26.7%**) and *Klebsiella pneumoniae*(**13.3%**). Earlier studies on biliary microbiology have consistently reported *E. coli* as the predominant pathogen in bile cultures [20,23]. The increased proportion of non-fermenting organisms such as *Pseudomonas* has been associated with hospital exposure, prior antibiotic use, and endoscopic instrumentation, particularly ERCP [24]. These findings strongly support the concept of **ascending infection and duodeno-biliary reflux**, especially after endoscopic sphincterotomy, as described in earlier experimental and clinical studies [25].

Antibiotic sensitivity analysis in this study demonstrated highest sensitivity to **carbapenems (93.3%)**, followed by **amikacin (80.0%)** and **piperacillin–tazobactam (73.3%)**, while sensitivity to third-generation cephalosporins and fluoroquinolones was considerably lower. Contemporary studies have highlighted increasing antimicrobial resistance among biliary pathogens, particularly in tertiary care settings and post-ERCP patients [24,26]. The antibiotic sensitivity pattern observed in the present study aligns with recent reports that advocate escalation to broader-spectrum antibiotics in complicated gallstone disease and post-ERCP infections, while reserving narrower agents for uncomplicated cases [20,24].

A key surgical outcome in the present study was the significant association between bile contamination and operative difficulty. Difficult cholecystectomy was encountered in **53.3%** of cases overall, with the highest frequency in post-ERCP patients (**84.0%**), followed by cholecystitis patients (**52.0%**), and the lowest in uncomplicated gallstones (**24.0%**). This association was statistically significant ($\chi^2 = 18.11$, $p = 0.0001$). Earlier surgical series have reported similar findings, where prior ERCP emerged as a strong predictor of dense adhesions, distorted anatomy, and difficult dissection during cholecystectomy [18,19]. The concurrence of high bile culture positivity and increased surgical difficulty in Group C further supports the role of bacterial colonisation and chronic inflammation in complicating surgical planes.

Overall, when compared with existing literature in chronological context, the present study reinforces the concept that bile is largely sterile in uncomplicated gallstones, variably contaminated in cholecystitis, and heavily colonised following ERCP. The predominance of enteric organisms, the antibiotic sensitivity profile, and the strong correlation with operative difficulty underscore the importance of microbiological evaluation and risk-based antibiotic selection in managing gallstone disease.

CONCLUSION

This prospective study demonstrates that bacterial colonisation of bile is strongly associated with the clinical presentation of gallstone disease and prior biliary interventions. Bile was largely sterile in patients with asymptomatic or uncomplicated gallstones, variably contaminated in patients with acute or chronic cholecystitis, and highly contaminated in post-ERCP patients. Enteric gram-negative organisms, particularly *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, were the predominant isolates, supporting the role of ascending duodeno-biliary reflux, especially following sphincterotomy. The antibiotic sensitivity pattern showed maximum susceptibility to carbapenems, followed by amikacin and piperacillin–tazobactam, indicating the need for tailored antibiotic selection in high-risk groups. A significant association was observed between bile contamination and increased intraoperative difficulty, particularly in post-ERCP patients. Overall, the study highlights the importance of microbiological assessment of bile and risk-stratified perioperative management in patients undergoing cholecystectomy.

LIMITATIONS

The study had certain limitations. The sample size was relatively modest and derived from a single tertiary care centre, which may limit the generalisability of the findings to other settings. Anaerobic cultures and molecular techniques for microbiome analysis were not performed, which could have underestimated the true spectrum of biliary microorganisms. Prior antibiotic exposure in some patients during earlier episodes of biliary illness may have influenced bile culture results. Additionally, long-term postoperative outcomes such as surgical site infections and late complications were not evaluated, restricting the assessment to perioperative and intraoperative correlations.

RECOMMENDATIONS

Based on the findings of this study, routine bile culture is not recommended for patients with uncomplicated gallstone disease due to the high likelihood of sterile bile. However, bile culture and sensitivity testing should be strongly considered in patients with acute cholecystitis and especially in those with a history of ERCP, as these groups demonstrate a high prevalence of bacterial contamination and increased operative difficulty. Empirical antibiotic therapy in such high-risk patients should cover gram-negative enteric organisms, with carbapenems or piperacillin–tazobactam reserved for severe or post-ERCP cases based on local sensitivity patterns. Larger multicentric studies incorporating anaerobic cultures and molecular diagnostic techniques are recommended to further characterise biliary microbiota and refine antibiotic protocols. Early elective cholecystectomy after ERCP may also be considered to reduce inflammation-related surgical difficulty.

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