



CLINICAL ASPECTS OF BILE CULTURE AND ANTIBIOTIC SENSITIVITY IN PATIENTS UNDERGOING CHOLECYSTECTOMY

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ABSTRACT **Background:** Cholecystectomy is one of the most commonly performed surgical procedures, most frequently indicated for gallstone disease. Bacterial colonization of bile (bacteriobilia) may occur in patients with gallbladder pathology and may contribute to postoperative infectious complications. Identification of the microbiological flora present in bile is important for guiding appropriate antibiotic prophylaxis. **Aim:** To assess Clinical Aspects of Bile Culture and Antibiotic Sensitivity in Patients Undergoing Cholecystectomy

Objectives

1. To evaluate the microbiological profile of bile obtained from the gallbladder in patients undergoing cholecystectomy.
2. To determine the appropriate antibiotic for preoperative prophylaxis based on the microbiological profile of bile.

Methods: This hospital-based prospective observational study was conducted in the Department of General Surgery at Tezpur Medical College and Hospital over six months. Bile samples were aspirated intraoperatively before cholecystectomy and sent for culture and antibiotic sensitivity testing **Results:** Bacteriobilia was observed in 32% of patients. *Escherichia coli* was the most common organism isolated, followed by *Klebsiella* species. Most isolates were sensitive to Amikacin and Piperacillin-Tazobactam. **Conclusion:** Identification of bile microbiology and antibiotic sensitivity patterns may help guide appropriate prophylactic antibiotic therapy and reduce postoperative infectious complications.

KEYWORDS : Bacteriobilia, Cholecystectomy, Bile Culture, Antibiotic Sensitivity, Gallstone Disease.

INTRODUCTION

Gallstone disease is one of the most common hepatobiliary disorders worldwide and represents a major indication for cholecystectomy. The prevalence of gallstones in the adult population is estimated to be approximately 10–15%, with higher incidence observed among females and elderly individuals. Cholecystectomy remains the definitive treatment for symptomatic gallstone disease and its complications, including acute cholecystitis, choledocholithiasis, biliary obstruction, and cholangitis. With the widespread adoption of laparoscopic techniques, cholecystectomy has become one of the most frequently performed surgical procedures in modern surgical practice (1)

Under normal physiological conditions, bile within the biliary tract is sterile. This sterility is maintained by several protective mechanisms including continuous bile flow, bacteriostatic properties of bile salts, and the barrier function of the sphincter of Oddi. However, when these protective mechanisms are disrupted due to gallstones, biliary obstruction, or inflammation, bacterial colonization of bile, known as bacteriobilia, may occur (2). Bile stasis caused by obstruction provides a favourable environment for bacterial proliferation within the biliary tract.

Several studies have evaluated the microbiological profile of bile in patients with gallstone disease. Kapoor et al. reported that bacteriobilia was present in approximately 30–40% of patients undergoing cholecystectomy, with *Escherichia coli* being the most commonly isolated organism followed by *Klebsiella* species.(3) Similarly, Brook observed that Gram-negative enteric organisms such as *E. coli*, *Klebsiella*, and *Proteus* constitute the predominant pathogens isolated from infected bile, while organisms such as *Enterococcus* and *Pseudomonas* are less frequently encountered.(4) Chang et al. also demonstrated that bacteriobilia may occur in 20–50% of patients undergoing cholecystectomy, and patients with positive bile cultures may have a higher risk of postoperative infectious complications.(5)

Prophylactic antibiotics are routinely administered in patients undergoing cholecystectomy to reduce postoperative infectious complications. However, antibiotic prophylaxis does not necessarily sterilize bile, and bacteriobilia may persist despite adequate antibiotic concentrations in bile.(6) Therefore, knowledge of the microbiological flora of bile and the antibiotic sensitivity pattern of isolated organisms is important for selecting appropriate prophylactic antibiotics and improving surgical outcomes.

Hence, the present study was undertaken to evaluate the microbiological profile of bile in patients undergoing cholecystectomy and to determine appropriate antibiotics for prophylaxis based on culture and sensitivity patterns.

Aim

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Objectives

- To evaluate the microbiological profile of bile obtained from the gallbladder in patients undergoing cholecystectomy.
- To determine the appropriate antibiotic for preoperative prophylaxis based on the microbiological profile of bile.

MATERIALS AND METHODS

Study Name: Clinical Aspects of Bile Culture and Antibiotic Sensitivity in Patients Undergoing Cholecystectomy. This study is a hospital-based prospective observational study. The study was conducted in the Department of General Surgery at Tezpur Medical College and Hospital, Tezpur, Assam.

Study Period - 6 months (1/7/2024- 31/12/2024)

Study Population: All patients admitted to the Department of General Surgery and undergoing cholecystectomy during the study period following the inclusion and exclusion criteria.

Sample Size: 100

Inclusion Criteria

- Patients undergoing open or laparoscopic cholecystectomy

Exclusion Criteria

- Immunocompromised patients
- Patients with sepsis
- History of cholangitis
- Patients who have undergone ERCP

METHODOLOGY

All patients admitted to Tezpur Medical College and Hospital who underwent cholecystectomy between 1 July 2024 and 31 December 2024 were included in the study.

Detailed demographic information including name, age, and sex was recorded. A comprehensive clinical history was obtained and a thorough physical examination was performed. Routine laboratory investigations were conducted as part of the preoperative evaluation.

Radiological investigations such as ultrasonography, computed tomography (CT), and magnetic resonance cholangiopancreatography (MRCP) were performed when required to confirm gallbladder pathology prior to surgery.

During surgery, approximately 2 mL of bile was aspirated from the gallbladder using a sterile syringe before performing cholecystectomy. The bile sample was collected under strict aseptic precautions and transferred immediately into a sterile container.

The collected bile sample was transported to the microbiology laboratory for analysis. The specimen was examined to determine whether it was sterile or contained bacterial growth. In cases where bacterial growth was detected, the organism was identified using standard microbiological techniques.

Antibiotic sensitivity testing was performed to determine the susceptibility of the isolated organisms to commonly used antimicrobial agents.

Statistical Analysis

The collected data were analyzed using descriptive statistical methods. Results were presented in the form of tables, charts, and figures wherever appropriate. Statistical assistance was obtained from a statistician for analysis.

Ethical Review

The protocol was submitted to the institutional ethical committee and the study was conducted only after approval from the same committee vide No 2024/074/TMC&H, issued on 22/06/2024.

RESULTS

A total of 100 patients undergoing cholecystectomy were included in the study conducted at the Department of General Surgery, Tezpur Medical College and Hospital.

1. Demographic Profile of Patients

Table 1: Age Distribution of Patients

Age Group (years)	Number of Patients	Percentage
20-29	8	8%
30-39	20	20%
40-49	35	35%
50-59	25	25%
≥60	12	12%
Total	100	100%

Most patients were in the 40-49 year age group (35%).

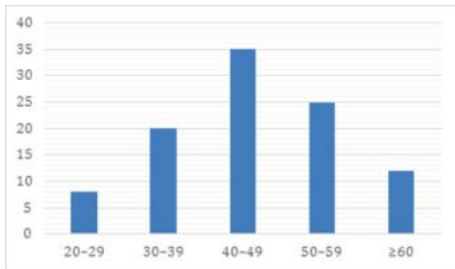
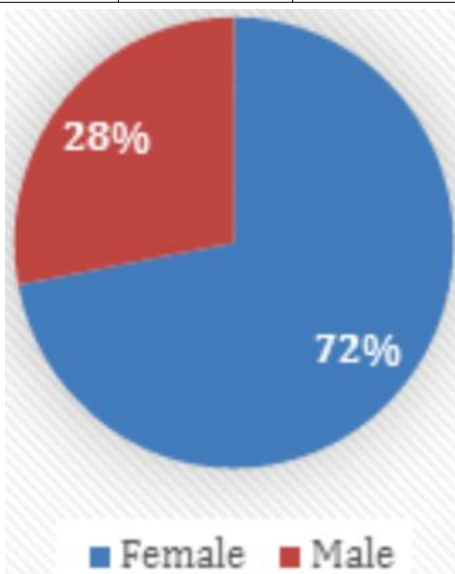


Table 2: Sex Distribution

Sex	Number	Percentage
Female	72	72%
Male	28	28%
Total	100	100%

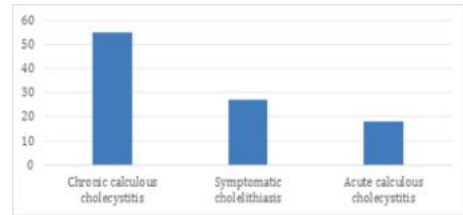


The sex distribution of the study population is shown in Table 2. Out of the 100 patients included in the study, 72 were females and 28 were males, giving a female-to-male ratio of approximately 2.6:1. The higher prevalence of gallstone disease among females observed in the present study is consistent with the known epidemiological pattern of gallstone disease.

2. Clinical Diagnosis

Table 3: Indication for Cholecystectomy

Diagnosis	Number	Percentage
Chronic calculous cholecystitis	55	55%
Symptomatic cholelithiasis	27	27%
Acute calculous cholecystitis	18	18%
Total	100	100%

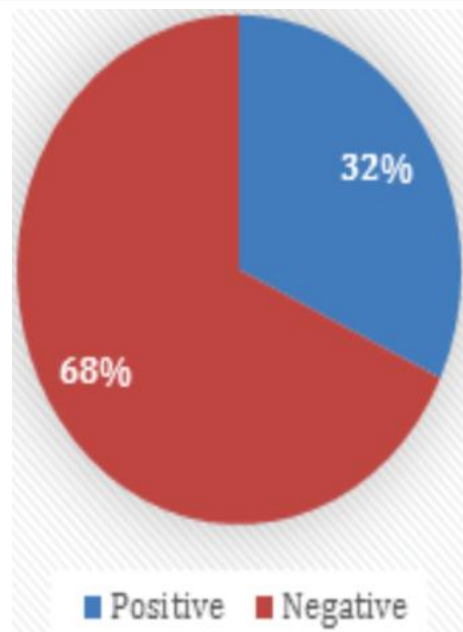


The distribution of patients according to the clinical diagnosis is shown in Table 3. The most common indication for cholecystectomy in the present study was chronic calculous cholecystitis, which was observed in 55% of patients.

4. Incidence of Bacteriobilia

Table 5: Bile Culture Results

Bile Culture	Number	Percentage
Positive	32	32%
Negative	68	68%
Total	100	100%

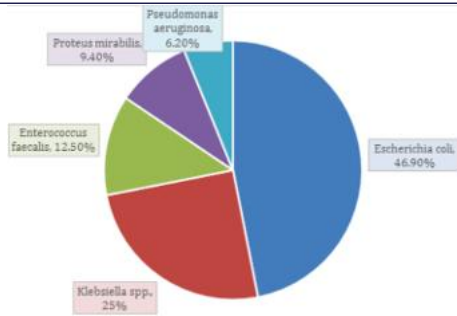


Bile samples obtained during surgery were subjected to microbiological analysis. The results of bile culture are shown in Table 5. Out of the 100 bile samples analyzed, 32 samples showed positive bacterial growth, indicating the presence of bacteriobilia, while 68 samples were sterile.

5. Microbiological Profile of Bile

Table 6: Organisms Isolated from Bile Culture

Organism	Number	Percentage
Escherichia coli	15	46.9%
Klebsiella spp.	8	25%
Enterococcus faecalis	4	12.5%
Proteus mirabilis	3	9.4%
Pseudomonas aeruginosa	2	6.2%
Total	32	100%



Graph: Distribution of Organisms Isolated from Bile Culture

Among the 32 positive bile cultures, the most frequently isolated organism was *Escherichia coli*, which accounted for 15 cases (46.9%). The second most common organism was *Klebsiella* species, isolated in 8 cases (25%). Other organisms isolated included *Enterococcus faecalis* (12.5%), *Proteus mirabilis* (9.4%), and *Pseudomonas aeruginosa* (6.2%).

The results demonstrate that Gram-negative enteric bacteria were the predominant organisms isolated from bile samples, with *Escherichia coli* being the most common pathogen.

6. Antibiotic Sensitivity Pattern

Table 7: Antibiotic Sensitivity of Isolated Organisms

Antibiotic	Sensitive Isolates (n=32)	Percentage
Amikacin	26	81.3%
Piperacillin–Tazobactam	24	75%
Cefoperazone–Sulbactam	22	68.8%
Meropenem	20	62.5%

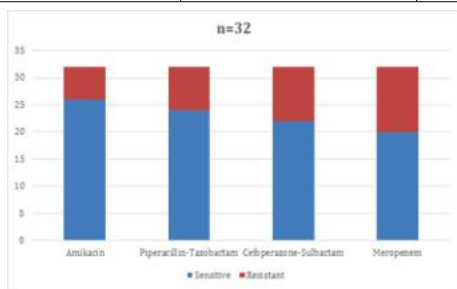


Figure 2: Antibiotic Sensitivity Pattern of Isolates

Antibiotic susceptibility testing of the isolated organisms revealed that Amikacin showed the highest sensitivity (81%), followed by Piperacillin–Tazobactam (75%). Other antibiotics such as Cefoperazone–Sulbactam (69%) and Meropenem (63%) also demonstrated good sensitivity.

These findings suggest that Amikacin and Piperacillin–Tazobactam may be effective antibiotics for prophylactic or therapeutic use in biliary infections associated with gallstone disease.

7. Postoperative Infection

Table 8: Postoperative Infection in Relation to Bile Culture

Bile Culture	Infection Present	Infection Absent	Total
Positive	4	28	32
Negative	1	67	68

Postoperative infectious complications were observed in 5 patients in the study. Among these, 4 patients had positive bile cultures, while 1 patient had a sterile bile culture.

This observation suggests that patients with bacteriobilia may have a higher risk of postoperative infection, highlighting the clinical significance of identifying bacterial colonization of bile in patients undergoing cholecystectomy.

Association Between Demographic and Clinical Variables with Bile Culture Positivity

To evaluate factors associated with bacteriobilia, the relationship between age, sex, and clinical diagnosis with bile culture positivity was analyzed using the Chi-square test.

Table 9: Association Between Age Group and Bile Culture Positivity

Age Group (years)	Culture Positive	Culture Negative	Total	Positivity (%)
20–29	1	7	8	12.5
30–39	5	15	20	25.0
40–49	10	25	35	28.6
50–59	9	16	25	36.0
≥60	7	5	12	58.3
Total	32	68	100	32.0

Chi-square = 10.62

p = 0.031

Bile culture positivity increased with advancing age, with the highest positivity observed among patients aged 60 years and above. The association between age group and bile culture positivity was statistically significant.

Table 10: Association Between Diagnosis and Bile Culture Positivity

Diagnosis	Culture Positive	Culture Negative	Total	Positivity (%)
Chronic calculous cholecystitis	16	39	55	29.1
Symptomatic cholelithiasis	5	22	27	18.5
Acute calculous cholecystitis	11	7	18	61.1

Chi-square = 13.87

p = 0.001

Bacteriobilia was significantly more common in patients with acute calculous cholecystitis compared to other diagnostic groups. The association between clinical diagnosis and bile culture positivity was statistically significant.

Table 11: Predictors of Bacteriobilia (Odds Ratio Analysis)

Variable	Culture Positive	Culture Negative	Odds Ratio (OR)	95% CI	p-value
Age ≥50 years	16	21	2.13	1.01 – 4.51	0.046
Acute calculous cholecystitis	11	7	5.08	1.82 – 14.15	0.002

Patients aged ≥50 years had approximately 2.1 times higher odds of bacteriobilia compared to younger patients.

This association was statistically significant (p = 0.046).

Patients with acute calculous cholecystitis had significantly higher odds of bacteriobilia compared to other diagnostic groups (OR = 5.08, p = 0.002).

DISCUSSION

The present study was conducted to evaluate the microbiological profile of bile and antibiotic sensitivity patterns in patients undergoing cholecystectomy. The findings of the study were analyzed with respect to demographic characteristics, bacteriological profile of bile, antibiotic sensitivity patterns, and postoperative wound infection rates, and were compared with findings reported in previous studies.

Age Distribution

In the present study, the majority of patients belonged to the 40–49 year age group, indicating that gallstone disease is more common among middle-aged individuals. Similar age distribution has been reported in several previous studies, which have shown that gallstone disease most frequently affects individuals in the fourth and fifth decades of life.

Study	Age group with majority cases (years)
Brook I., (1989)(4)	53
Méndez Sánchez et al. (2005)(7)	51
Chang WT et al.(2002)(5)	41
West et al. (2009)(8)	49.8±21.6
Velázquez-Mendoza et al. (2010)(9)	50.45
Chang WT et al.(2002)(5)	53.7 ± 17.5
Pimpale R et al.(2019)(10)	45.03yrs ± 13.59
Present study	40–49

Sex Distribution

The present study demonstrated a clear female predominance, with females accounting for 72% of the study population. Similar findings have been reported in previous studies, indicating that gallstone disease occurs more frequently in women.

Study	Female
Brook I., (1989)(4)	34.4%
Chang WT et al.(2002)(5)	67.6%
West et al. (2009)(8)	71.5%
Velázquez-Mendoza et al. (2010)(9)	70%
Chang WT et al.(2002)(5)	73%
Gyedu et al. (2015)(11)	Women RR 1.45
Pimpale R et al.(2019)(10)	68.89%
Present study(2025)	72%

Bacteriological Profile of Bile

In the present study, *Escherichia coli* was the most frequently isolated organism (46%), followed by *Klebsiella* species (25%) and *Enterococcus* species (12.5%). Similar findings have been reported in previous studies, which show that Gram-negative enteric bacteria are the predominant organisms isolated from bile cultures. The predominance of these organisms may be due to the ascending spread of intestinal flora into the biliary tract. Conditions such as gallstones, biliary obstruction, and inflammation may impair normal bile flow and facilitate bacterial colonization of bile.

Study	Culture Positive organisms in Bile			
	E.Coli	Klebsiella	Enterococcus	Others
Brook I., (1989)(4)	24%	10.1%	14.6%	
Chang WT et al.(2002)(5)	36%	15%	6%	
Capoor MR et al., 2008(3)	11, 29.7%	10, 27%	-	
Kaya et al. (2012)(12)	28.2%	-	-	<i>Pseudomonas</i> (17.3%) <i>Stenotrophomonas maltophilia</i> (15.2%)
Li et al (2018)(13)	53.18%	10.11%	10.49%	
Kumar et al. (2018) (14)	51.16%	20.93%	-	<i>Pseudomonas</i> (9.30%) <i>Salmonella</i> (6.98%) <i>Citrobacter</i> (6.98%) <i>Proteus</i> (4.65%).
Zhao et al. (2022) (15)	30%	15.7%	12.1%	<i>Pseudomonas aeruginosa</i> (4.3%) <i>Enterobacter</i> (4.3%)
Present study (2025) (n=32)	46%	25%	12.5%	

Antibiotic Sensitivity Pattern

The antibiotic susceptibility pattern in the present study showed the highest sensitivity to Amikacin (81.3%), followed by Piperacillin–Tazobactam (75%), Cefoperazone–Sulbactam (68.8%), and Meropenem (62.5%). Similar findings have been reported in previous studies, which demonstrate high sensitivity of biliary pathogens to these antibiotics.

Study	Antibiotic sensitive isolates				
	Amikacin	Piperacillin–Tazobactam	Cefoperazone–Sulbactam	Meropenem	Imipenem
Abeyasuriya et al. (2008) (16)	100%	-		100%	100%
Kaya et al. (2012)(12)	86%	61%	-	86%	79%
Amarenddra et al. (1015)(17)	65.51%	62.07%	-	86%	82%
Li et al (2018)(13)	88.46%	85.58%	92.31%	-	-
Zhao et al. (2022) (15)	~99%	95%	95%	-	97.5%
Kumar et al. (2018) (14)	70.45%	61.36%	-	72.73%	79.55%

Present study(2025)	81.3%	75%	68.8%	62.5%	-
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Postoperative Wound Infection

study	Wound infection in	
	Bile Culture positive	Bile culture negative
Wolloch Y et al (1977)(18)	29%	19.6%
Garibaldi et al. (1986) (19)	11%	2%
Gold deutch et al.(1996)(20)	0%	1%
Velázquez-Mendoza et al. (2010)(9)	23%	7.8%
K S et al. (2014)(21)	32.2%	0%
Reddy et al. (2021) (22)	33%	13.7%
Present study (2025)(18)	12.5%	1.4%

In the present study, postoperative wound infection was more common in patients with positive bile cultures (12.5%) compared to those with sterile bile (1.4%). Similar findings have been reported in previous studies, suggesting that bacteriobilia may increase the risk of postoperative infectious complications following cholecystectomy. Identification of bacterial organisms in bile and their antibiotic sensitivity patterns may therefore help guide appropriate prophylactic antibiotic therapy and reduce postoperative morbidity.

Overall Interpretation

Overall, the findings of the present study are largely consistent with previous literature. Gallstone disease predominantly affected middle-aged females, and Gram-negative enteric organisms, particularly *Escherichia coli*, were the most common pathogens isolated from bile cultures. Antibiotics such as Amikacin and Piperacillin–Tazobactam demonstrated high sensitivity against the isolated organisms, suggesting their potential role in the management of biliary infections. Furthermore, the presence of bacteriobilia was associated with a higher incidence of postoperative wound infection, highlighting the clinical importance of identifying bacterial colonization of bile in patients undergoing cholecystectomy.

Summary

My study “Clinical Aspects of Bile Culture and Antibiotic Sensitivity in Patients Undergoing Cholecystectomy” is a Hospital Based prospective observational study, done in the Department of General Surgery, Tezpur Medical College and Hospital for 6 months from 1/7/2024- 31/12/2014

The Key Findings were:

- The majority of patients were in the 40–49 year age group (35%), followed by 50–59 years (25%), indicating that gallstone disease was most common among middle-aged individuals. A clear female predominance was observed, with females accounting for 72% of the study population.
- The most common clinical diagnosis was chronic calculous cholecystitis (55%), followed by symptomatic cholelithiasis (27%) and acute calculous cholecystitis (18%). Laparoscopic cholecystectomy was the most frequently performed procedure, accounting for 90% of surgeries.
- Bile culture analysis revealed that 32% of patients had bacteriobilia, while 68% of bile samples were sterile. Among the positive cultures, Gram-negative enteric organisms predominated. The most commonly isolated organism was *Escherichia coli* (46.9%), followed by *Klebsiella* species (25%), *Enterococcus faecalis* (12.5%), *Proteus mirabilis* (9.4%), and *Pseudomonas aeruginosa* (6.2%).
- Antibiotic susceptibility testing of the 32 bacterial isolates demonstrated the highest sensitivity to Amikacin (81.3%), followed by Piperacillin–Tazobactam (75%), Cefoperazone–Sulbactam (68.8%), and Meropenem (62.5%).
- Postoperative infections were observed more frequently in patients with positive bile cultures, suggesting a possible association between bacteriobilia and postoperative infectious complications.
- Logistic regression analysis demonstrated that acute calculous cholecystitis was a significant predictor of bacteriobilia (OR = 5.08, p = 0.002). Age ≥50 years was also associated with higher odds of positive bile culture (OR = 2.13, p = 0.046).

CONCLUSION

The present study demonstrated that bacteriobilia was present in 32% of patients undergoing cholecystectomy. Gram-negative enteric organisms were the predominant pathogens isolated from bile, with *Escherichia coli* being the most common organism.

Antibiotic sensitivity patterns indicated that Amikacin and Piperacillin-Tazobactam were the most effective antimicrobial agents against the isolated organisms. Knowledge of the microbiological profile of bile and its antibiotic sensitivity pattern can help guide appropriate prophylactic antibiotic therapy in patients undergoing cholecystectomy.

Routine bile culture during cholecystectomy may therefore be useful in identifying bacterial colonization and in preventing postoperative infectious complications through targeted antimicrobial therapy.

This limitation of my study is that It was a single-center, hospital-based study with a relatively small sample size, which may limit the generalizability of the findings to the wider population.

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