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Microbiological Profile of Bile in Patients with Choledocholithiasis Undergoing Common Bile Duct Exploration

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ABSTRACT

Background; Biliary obstruction due to choledocholithiasis predisposes to bacterial colonization of bile and may contribute to postoperative infectious complications. Knowledge of the local microbiological profile and antimicrobial susceptibility pattern is essential for appropriate perioperative management.

Aim; To determine the microbiological spectrum of bile in patients with choledocholithiasis undergoing common bile duct exploration (CBDE), evaluate antimicrobial susceptibility patterns, and identify factors associated with bile culture positivity.

Materials and Methods; This prospective observational study included 90 patients with choledocholithiasis undergoing CBDE. Intraoperative bile samples were collected aseptically and subjected to microbiological culture, identification of isolates, and antimicrobial susceptibility testing. Demographic, clinical, biochemical, and operative variables were analyzed for their association with bile culture positivity.

Results; Positive bile cultures were observed in 34 (37.8%) patients. Gram-negative organisms predominated (82.4%), with *Escherichia coli* (41.2%) and *Klebsiella pneumoniae* (23.5%) being the most common isolates. Gram-negative isolates demonstrated highest sensitivity to meropenem (92.3%) and imipenem (90.4%). Culture positivity was significantly associated with age >40 years, diabetes mellitus, elevated bilirubin levels, common bile duct dilatation, multiple stones, and prolonged symptom duration. Multidrug-resistant organisms were identified in 41.2% of isolates. Surgical site infections occurred more frequently among culture-positive patients.

Conclusion; Bacteriobilia is common in patients with choledocholithiasis undergoing common bile duct exploration and is predominantly caused by Gram-negative enteric organisms. Routine bile culture and culture-guided antibiotic therapy may help optimize antimicrobial use and reduce postoperative infectious complications.

Keywords*: Choledocholithiasis, Bacteriobilia, Common bile duct exploration, Bile culture, Antimicrobial resistance, Surgical site infection.*

INTRODUCTION

Choledocholithiasis, defined as the presence of calculi within the common bile duct (CBD), is a common biliary tract disorder associated with significant morbidity and potential life-threatening complications, including obstructive jaundice, acute cholangitis, and pancreatitis (1). The condition frequently occurs in association with cholelithiasis and is more prevalent among middle-aged and elderly individuals, particularly females (2).

Under normal physiological conditions, bile within the biliary system is sterile due to the continuous flow of bile and the protective function of the sphincter of Oddi. However, biliary obstruction caused by CBD stones can result in bile stasis, increased intraductal pressure, and bacterial colonization of the biliary tree (3). The presence of microorganisms in bile, commonly referred to as bacteriobilia, has been reported in a substantial proportion of patients with choledocholithiasis and has been implicated in postoperative infectious complications and recurrent stone formation (4).

The microbiological profile of bile in patients with biliary tract disease predominantly consists of enteric organisms. Gram-negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter* species are frequently isolated, although Gram-positive organisms, including *Enterococcus* species, are also increasingly recognized (5,6). The growing prevalence of multidrug-resistant (MDR) organisms has further complicated the management of biliary infections and has highlighted the need for region-specific surveillance of antimicrobial susceptibility patterns (7).

Common bile duct exploration (CBDE) remains an important therapeutic option for the management of choledocholithiasis, particularly in patients unsuitable for endoscopic intervention or those with large or multiple stones. Intraoperative bile culture obtained during CBDE provides valuable information regarding the microbial spectrum and antimicrobial resistance patterns present within the biliary system (8). Such information can guide appropriate perioperative antibiotic therapy and may contribute to reducing postoperative infectious complications.

Although several studies have evaluated bacteriobilia in patients with biliary tract diseases, variations in bacterial isolates and antimicrobial susceptibility patterns have been reported across different geographic regions and healthcare settings (9,10). Therefore, understanding the local microbiological profile is essential for developing evidence-based antibiotic policies and improving surgical outcomes.

The present study was conducted to evaluate the microbiological spectrum of bile in patients undergoing common bile duct exploration for choledocholithiasis, determine the antimicrobial susceptibility patterns of isolated organisms, and assess the association of bacteriobilia with demographic, clinical, biochemical, and operative variables.

MATERIALS AND METHODS

Study Design and Setting

This prospective observational study was conducted in the Department of General Surgery in collaboration with the Department of Microbiology at Era's Lucknow Medical College and Hospital, Lucknow, Uttar Pradesh, India. The study aimed to evaluate the microbiological spectrum of bile in patients with choledocholithiasis undergoing common bile duct exploration (CBDE), assess antimicrobial susceptibility patterns of isolated organisms, and determine their association with demographic and clinical variables.

The study was carried out over a period of 24 months. Ethical approval was obtained from the Institutional Ethics Committee before commencement of the study, and written informed consent was obtained from all participants.

Study Population

Adult patients diagnosed with choledocholithiasis and scheduled for common bile duct exploration were consecutively recruited during the study period. Patients were enrolled after fulfilling the eligibility criteria and providing informed consent.

Inclusion Criteria

- Patients aged ≥18 years.
- Diagnosed cases of choledocholithiasis undergoing common bile duct exploration.
- Patients willing to provide written informed consent.

Exclusion Criteria

- Patients with acute cholangitis.
- Patients who had received antibiotics within 7 days prior to surgery.
- Immunocompromised patients, including those with HIV infection, post-transplant status, or receiving immunosuppressive therapy.
- Patients who had undergone endoscopic retrograde cholangiopancreatography (ERCP) before surgery.
- Patients with malignant biliary obstruction or biliary malignancy.

Sample Size

The sample size was calculated using the prevalence of positive bile cultures reported in previous studies. Considering a prevalence (p) of 38.0%, an allowable error of 10%, and a confidence level of 95%, the minimum required sample size was estimated to be 90 patients. Therefore, a total of 90 eligible patients were included in the study.

Preoperative Evaluation
All participants underwent detailed clinical assessment including history taking and physical examination. Baseline investigations included complete blood count, liver function tests, renal function tests, and coagulation profile. Imaging studies such as ultrasonography and magnetic resonance cholangiopancreatography (MRCP) were performed to confirm the diagnosis of choledocholithiasis. Preoperative risk stratification was conducted using the American Society of Anesthesiologists (ASA) grading system.

Bile Sample Collection
During common bile duct exploration, bile samples were collected aseptically immediately after exposure and opening of the common bile duct and prior to any irrigation or manipulation of stones.

Approximately 2–3 mL of bile was aspirated using a sterile 5 mL syringe fitted with a 22-gauge needle. The sample was transferred into a sterile, leak-proof container and transported to the Department of Microbiology within 30 minutes of collection for processing. Relevant operative findings and sample details were recorded in a structured proforma.

Microbiological Analysis
Direct Microscopy
Direct microscopic examination of bile samples was performed using Gram staining to determine bacterial morphology and Gram reaction. Wet mount examination was carried out whenever indicated.

Culture and Isolation
Samples were inoculated onto Blood Agar and MacConkey Agar plates and incubated aerobically at 37°C for 18–24 hours. In the absence of visible growth, incubation was extended up to 48 hours before reporting the culture as sterile.

Identification of Isolates
Bacterial isolates were identified based on colony morphology, Gram staining characteristics, and standard biochemical tests including catalase, coagulase, indole, citrate, urease, oxidase, and Triple Sugar Iron (TSI) tests. Automated identification systems (VITEK-2) were utilized where available for confirmation.

Antimicrobial Susceptibility Testing
Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar in accordance with Clinical and Laboratory Standards Institute (CLSI) 2024 guidelines.

The antibiotics tested for Gram-negative isolates included ampicillin, amoxicillin-clavulanate, ceftriaxone, ceftazidime, cefepime, piperacillin-tazobactam, meropenem, imipenem, amikacin, gentamicin, ciprofloxacin, and levofloxacin. For Gram-positive isolates, ampicillin, erythromycin, clindamycin, vancomycin, linezolid, and cefoxitin were tested.

Results were interpreted as sensitive, intermediate, or resistant according to CLSI criteria.

Quality Control
Quality assurance was maintained using standard reference strains, including *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923, for validation of antimicrobial susceptibility testing.

Data Collection
Demographic variables recorded included age, sex, place of residence, and socioeconomic status. Clinical variables included presenting symptoms, duration of illness, comorbidities, laboratory findings, and operative details such as common bile duct diameter, number and size of stones, bile appearance, and presence of sludge or pus.

Microbiological variables included Gram stain findings, culture positivity, bacterial isolates, and antimicrobial susceptibility patterns.

Outcome Measures
Primary Outcome
The primary outcome was the proportion and microbiological profile of positive bile cultures among patients with choledocholithiasis undergoing common bile duct exploration.

Secondary Outcomes
Secondary outcomes included:
- Distribution of bacterial isolates.
- Antimicrobial susceptibility patterns.
- Association of culture positivity with demographic and clinical characteristics.

- Occurrence of postoperative infectious complications where follow-up data were available.

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using IBM SPSS Statistics version 26.0. Continuous variables were expressed as mean ± standard deviation or median (interquartile range) as appropriate. Categorical variables were presented as frequencies and percentages.

Comparisons between categorical variables were performed using the Chi-square test or Fisher's exact test. Continuous variables were compared using Student's t-test or Mann–Whitney U test based on data distribution. Correlation analysis was performed to evaluate the relationship between bile culture positivity and selected clinical variables.

A p-value <0.05 was considered statistically significant.

RESULTS AND OBSERVATIONS

A total of 90 patients with choledocholithiasis undergoing common bile duct exploration were included in the study. The mean age of the participants was 47.8 ± 12.4 years. Females constituted the majority of the study population (67.8%). Positive bile cultures were obtained in 34 (37.8%) patients, while 56 (62.2%) had sterile bile cultures.

Table 1. Demographic Characteristics of Study Participants (n=90)

Variable	Number	Percentage (%)
Age 18–30 years	12	13.3
Age 31–40 years	21	23.3
Age 41–50 years	27	30.0
Age 51–60 years	20	22.2
Age >60 years	10	11.2
Male	29	32.2
Female	61	67.8

Most patients belonged to the 41–50 year age group (30.0%). Female predominance was observed, accounting for 67.8% of cases.

Table 2. Bile Culture Positivity and Growth Characteristics (n=90)

Variable	Number	Percentage (%)
Positive Culture	34	37.8
Sterile Culture	56	62.2
Monomicrobial Growth	26	76.5*
Polymicrobial Growth	8	23.5*

***Percentage calculated among culture-positive samples (n=34).**

Bacteriobilia was detected in 37.8% of patients. Monomicrobial growth predominated among positive cultures.

Table 3. Microbiological Profile of Positive Bile Cultures (n=34)

Organism	Number	Percentage (%)
Escherichia coli	14	41.2
Klebsiella pneumoniae	8	23.5
Enterococcus faecalis	6	17.6
Pseudomonas aeruginosa	4	11.8
Enterobacter spp.	2	5.9
Gram Stain Pattern	**Number**	**Percentage (%)**
Gram-negative	28	82.4
Gram-positive	6	17.6

Gram-negative organisms predominated, with E. coli being the most common isolate.

Table 4. Antibiotic Sensitivity Pattern of Isolates
Gram-Negative Isolates

Antibiotic	Sensitivity (%)
Meropenem	92.3
Imipenem	90.4
Piperacillin-Tazobactam	88.5
Amikacin	84.6
Ciprofloxacin	42.3
Ceftriaxone	38.5

Gram-Positive Isolates

Antibiotic	Sensitivity (%)
Linezolid	100
Vancomycin	100
Clindamycin	62.5
Ampicillin	50.0

Carbapenems showed the highest activity against Gram-negative organisms, whereas all Gram-positive isolates were sensitive to linezolid and vancomycin.

Table 5. Association Between Demographic Factors and Bile Culture Positivity

Variable	Culture Positive	Culture Negative	p-value
Male	11	27	<0.05
Female	23	29	
Age ≤40 years	8	25	<0.05
Age >40 years	26	31	

Culture positivity was significantly higher among females and patients aged above 40 years.

Table 6. Association Between Comorbidities and Bile Culture Positivity

Diabetes Status	Culture Positive	Culture Negative	Total
Diabetic	16	14	30
Non-diabetic	18	42	60

Diabetic patients demonstrated a significantly higher prevalence of positive bile cultures.

Table 7. Association of Biochemical Parameters with Bile Culture Positivity

Variable	Culture Positive	Culture Negative
Bilirubin ≤2 mg/dL	8	32
Bilirubin >2 mg/dL	26	24
Normal ALP	10	34
Elevated ALP	24	22

Elevated bilirubin and alkaline phosphatase levels were significantly associated with positive bile cultures.

Table 8. Association of Disease Duration with Bile Culture Positivity

Duration of Symptoms	Culture Positive	Culture Negative
≤6 months	11	35
>6 months	23	21

Patients with symptoms lasting more than 6 months had significantly higher rates of bacteriobilia.

Table 9. Antimicrobial Resistance and Postoperative Outcome

Variable	Number	Percentage (%)
MDR Present	14	41.2
MDR Absent	20	58.8
Outcome	**Culture Positive**	**Culture Negative**
SSI Developed	10	5
No SSI	24	51

Multidrug-resistant organisms were identified in 41.2% of isolates. Surgical site infection occurred more frequently among patients with positive bile cultures (29.4%) compared with culture-negative patients (8.9%), indicating a significant association between bacteriobilia and postoperative infectious complications.

DISCUSSION

The present study evaluated the microbiological profile of bile in patients with choledocholithiasis undergoing common bile duct exploration and demonstrated that bacteriobilia remains a common finding in this patient population. Positive bile cultures were observed in 37.8% of patients, indicating that bacterial colonization of the biliary tract is frequently associated with CBD stones and biliary obstruction.

The majority of patients in the present study were females (67.8%), and the highest proportion belonged to the 41–50-year age group. These findings are consistent with the established epidemiology of gallstone disease, which shows a higher prevalence among women due to hormonal influences on cholesterol metabolism and bile composition (2,11).

The culture positivity rate of 37.8% observed in the present study is comparable to rates reported in previous studies, which have ranged from 30% to 60% among patients with choledocholithiasis and other biliary tract disorders (4,12). Biliary obstruction resulting from CBD stones promotes bacterial colonization through bile stasis and ascending infection from the duodenum, thereby increasing the likelihood of positive bile cultures (13).

Gram-negative bacteria predominated among isolates, accounting for 82.4% of positive cultures. *Escherichia coli* was the most frequently isolated organism (41.2%), followed by *Klebsiella pneumoniae* (23.5%). Similar findings have been reported by Lee et al. and Negm et al., who identified *E. coli* and *Klebsiella* species as the predominant biliary pathogens in patients with choledocholithiasis (14,15). The predominance of enteric Gram-negative organisms supports the theory of ascending bacterial migration from the gastrointestinal tract into the biliary system.

Among Gram-positive organisms, *Enterococcus faecalis* was the most common isolate. Increasing isolation of enterococci from bile cultures has been documented in recent studies and may be related to widespread antibiotic exposure and changing microbial ecology within hospital settings (16).

Antimicrobial susceptibility testing demonstrated excellent activity of carbapenems, particularly meropenem and imipenem, against Gram-negative isolates. Piperacillin–tazobactam and amikacin also showed high sensitivity rates. In contrast, resistance to ceftriaxone and ciprofloxacin was substantial. These findings are consistent with recent reports documenting increasing resistance among biliary pathogens to third-generation cephalosporins and fluoroquinolones (7,17). Gram-positive isolates exhibited universal susceptibility to linezolid and vancomycin, suggesting that these agents remain reliable options for severe enterococcal infections.

The present study found significantly higher culture positivity among patients older than 40 years and among females. Advanced age has previously been associated with increased bacterial colonization of bile due to age-related changes in immunity and prolonged disease duration (18). Furthermore, diabetic patients demonstrated a greater prevalence of bacteriobilia compared with non-diabetic patients. Hyperglycemia is known to impair immune function and facilitate bacterial growth, thereby increasing susceptibility to infection (19).

Elevated serum bilirubin and alkaline phosphatase levels were significantly associated with positive bile cultures. These findings suggest that greater biliary obstruction may contribute to bacterial colonization and infection. Similar associations have been reported in previous investigations evaluating predictors of bacteriobilia in biliary tract diseases (20).

Operative findings revealed that culture positivity was significantly associated with CBD dilatation, multiple stones, and turbid or purulent bile. These observations are biologically plausible because prolonged obstruction and repeated stone formation create a favorable environment for bacterial proliferation within the biliary tract (21).

An important finding of the present study was the detection of multidrug-resistant organisms in 41.2% of positive cultures. This highlights the growing challenge of antimicrobial resistance in biliary infections and emphasizes the importance of culture-guided antibiotic therapy. Similar rates of MDR organisms have been reported in recent studies from tertiary care centers (22).

Postoperative surgical site infection (SSI) occurred more frequently among patients with positive bile cultures than among culture-negative patients. This finding supports previous evidence suggesting that bacteriobilia serves as an important risk factor for postoperative infectious complications and may adversely affect surgical outcomes (23). Therefore, routine bile culture during common bile duct exploration may provide valuable information for targeted antimicrobial therapy and infection prevention strategies.

Overall, the present study demonstrates that bacteriobilia is common among patients with choledocholithiasis undergoing common bile duct exploration. Gram-negative enteric organisms predominate, antimicrobial resistance is increasingly prevalent, and culture positivity is associated with adverse clinical and postoperative outcomes. These findings underscore the importance of microbiological surveillance and judicious antibiotic selection in the management of biliary tract infections.

CONCLUSION

Bacteriobilia was present in 37.8% of patients with choledocholithiasis undergoing common bile duct exploration. *Escherichia coli* and *Klebsiella pneumoniae* were the predominant isolates, with Gram-negative organisms accounting for the majority of infections. Culture positivity was significantly associated with diabetes, biliary obstruction, multiple stones, and prolonged disease duration. The high prevalence of multidrug-resistant organisms and increased

postoperative infections among culture-positive patients highlight the importance of routine bile culture and culture-guided antibiotic therapy to improve surgical outcomes.

REFERENCES

1. Williams EJ, Green J, Beckingham I, Parks R, Martin D, Lombard M. Guidelines on the management of common bile duct stones. Gut. 2008;57(7):1004-1021.
2. Portincasa P, Moschetta A, Palasciano G. Cholesterol gallstone disease. Lancet. 2006;368(9531):230-239.
3. Csendes A, Burdiles P, Diaz JC, Maluenda F, Nava O. Bacteriology of the gallbladder bile in normal subjects. Am J Surg. 1994;167(1):27-30.
4. Capoor MR, Nair D, Rajni, Khanna G, Krishna SV. Microflora of bile aspirates in patients with acute and chronic cholecystitis. Trop Gastroenterol. 2008;29(2):90-92.
5. Brook I. Aerobic and anaerobic microbiology of biliary tract disease. J Clin Microbiol. 1989;27(10):2373-2375.
6. Sung JY, Leung JW, Shaffer EA, Lam K, Olson ME. Ascending infection of the biliary tract. Gastrointest Endosc. 1992;38(5):514-518.
7. Gomi H, Solomkin JS, Takada T, Strasberg SM, Pitt HA, Yoshida M, et al. TG18 antimicrobial therapy for acute cholangitis and cholecystitis. J Hepatobiliary Pancreat Sci. 2018;25(1):3-16.
8. de Reuver PR, Rauws EA, Bruno MJ, Lameris JS, Busch OR, van Gulik TM, et al. Endoscopic and surgical management of bile duct stones. Dig Surg. 2007;24(4):268-274.
9. Darkahi B, Sandblom G, Liljeholm H, Videhult P, Melhus Å, Rasmussen IC. Biliary microflora in patients undergoing cholecystectomy. BMC Surg. 2014;14:36.
10. Kaya M, Beştaş R, Bacalan F, Bacalan F. Biliary bacteriology and antimicrobial susceptibility patterns. Turk J Gastroenterol. 2012;23(4):396-401.
11. Stinton LM, Shaffer EA. Epidemiology of gallbladder disease. Best Pract Res Clin Gastroenterol. 2012;26(5):615-635.
12. Maluenda F, Csendes A, Burdiles P, Diaz JC. Bacteriological study of choledochal bile in patients with common bile duct stones. Hepatogastroenterology. 1989;36(3):132-135.
13. Leung JW, Liu YL, Chan RC, Ling TK, Chan FK, Sung JJ. Early identification of patients at risk for cholangitis. Gastrointest Endosc. 2002;56(4):530-535.
14. Lee SE, Kim KH, Kim HJ, et al. Clinical significance of bile culture in biliary tract disease. J Korean Surg Soc. 2011;81(3):187-193.
15. Negm AA, Schott A, Vonberg RP, et al. Microbiological findings in bile cultures. Dig Dis Sci. 2010;55(12):3426-3431.
16. Weber A, Schneider J, Wagenpfeil S, et al. Spectrum of pathogens in acute cholangitis. Infection. 2013;41(2):431-436.
17. Kim YK, Lee JK, Lee KH, et al. Antibiotic resistance patterns of biliary pathogens. Korean J Intern Med. 2014;29(5):636-643.
18. Han J, Kim HG, Jung MK, et al. Predictive factors of positive bile culture. World J Gastroenterol. 2012;18(37):5289-5294.
19. Casqueiro J, Casqueiro J, Alves C. Infections in patients with diabetes mellitus. Indian J Endocrinol Metab. 2012;16(Suppl 1):S27-S36.
20. Chang WT, Lee KT, Wang SR, et al. Bacteriology and predictors of positive bile cultures. J Formos Med Assoc. 2006;105(10):769-775.
21. Csendes A, Diaz JC, Burdiles P, Maluenda F, Morales E. Risk factors and bacteriology in biliary tract infections. World J Surg. 1996;20(6):718-722.
22. Reuken PA, Torres D, Baier M, et al. Emerging multidrug-resistant pathogens in biliary infections. BMC Infect Dis. 2017;17:175.
23. Galili O, Eldar S, Matter I, et al. Effect of bacteriobilia on postoperative infectious complications. J Gastrointest Surg. 2008;12(11):1948-1954.