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Original Research Article

Biliary microbial colonization and antibiotic susceptibility in chronic calculous cholecystitis: a prospective observational study

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ABSTRACT

Background: One of the most common indications of cholecystectomy in the world is chronic calculous cholecystitis. Despite the fact that bile is physiologically sterile, gallstone disease predisposes microbial colonization by bile stasis, mucosal damage, and ascending infection. Bile microorganisms can be a contributor to infectious postoperative complications and affect the choice of perioperative antibiotics. Since there are regional differences in microbial flora and resistance to antibiotics, institution-specific data are required in evidence-based antimicrobial practice.
Methods: This was a prospective observational study design, based in a hospital and carried out on 87 patients who were undergoing cholecystectomy as a treatment of chronic calculous cholecystitis at a tertiary care centre in Uttar Pradesh after gaining ethical permission. Aseptically, intraoperative collection of about 2 ml of bile was done and subjected to regular microbiological procedures. The bacterium was identified using Gram staining and biochemical techniques after being cultured on blood agar and MacConkey agar. To determine antibiotic susceptibility, the Kirby-Bauer disc diffusion method was used in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines.
Results: Of the 87 patients who took part in the research, 70 (or 80.4% of the total) had cholecystectomy by laparoscopy, 12 (13.8%) had the procedure done openly, and 5 (5.8% of the total) had to have the procedure changed to open because laparoscopy failed. Biles were observed to be positive in 20 (23) patients, and sterile bile in 67 (77) patients. The most common isolate was *Escherichia coli* (45%), then *Klebsiella pneumoniae* (25%), then *Enterococcus faecalis* (15%), and *Pseudomonas aeruginosa* (15%). *E. coli* was more sensitive to cefuroxime and ciprofloxacin, and *Klebsiella pneumoniae* were intermediate to piperacillin-tazobactam and meropenem. *Enterococcus faecalis* was found to be sensitive to vancomycin and linezolid whereas *Pseudomonas aeruginosa* was found to be more sensitive to amikacin and piperacillin–tazobactam. Culture-positive patients had higher chances of postoperative wound infection, but this was not statistically significant.
Conclusion: Biliary microbial colonization was observed in about the quarter of patients with chronic calculous cholecystitis with *Escherichia coli* being the most common isolate. The high degree of antibiotic susceptibility variability underscores the need to have local antibiograms to optimize the use of antimicrobials during perioperative periods. Routine bile culture can be useful in the high-risk patients that undergo cholecystectomy who are selected.

Keywords: Chronic calculous cholecystitis, Bile culture, Biliary microbial colonization, Antibiotic susceptibility, Cholecystectomy, Antimicrobial resistance

INTRODUCTION

Cholelithiasis (gallstone disease) is one of the most frequent gastrointestinal diseases in the world and a significant indication of abdominal surgery, especially cholecystectomy. Gallstones occur in 10-20 percent of the world population, and there is an increasing trend of prevalence in developing nations as a result of dietary and

lifestyle habits, obesity, and metabolic disorders. Regional difference in disease burden has also been reported in India, with a comparatively high burden in the north as opposed to the southern states, indicating the impact of dietary, environmental, and genetic factors.[1,2]

Chronic calculous cholecystitis is a frequent effect of continuous irritation and inflammation of the gallbladder wall by the presence of gallstones. Despite the majority of patients not being symptomatic, symptomatic disease often exhibits recurrent abdominal pain, dyspepsia and biliary colic, and may also result in complications such as empyema, cholangitis, pancreatitis or gallbladder perforation. The conclusive treatment modality of symptomatic disease is cholecystectomy, be it laparoscopic or open.[3]

Bile is usually regarded to be sterile under physiological conditions due to its constant flow, bacteriostatic state and the anatomical walls like the sphincter of Oddi. Nevertheless, the presence of gallstone disease changes the dynamics of the bile by stagnating the bile, inflammating and blocking the mucosal layer, and exposing patients to the colonization of the gallbladder bile by microorganisms. The entry of bacteria into the biliary system can take place by ascending duodenal infection, hematogenous distribution via the portal circulation, lymphatic distribution, or enterohepatic recirculation. Moreover, the presence of gallstones can serve as a focal point of microbial attachment and biofilm formation that enhance microbial maintenance and chronic inflammation.[4,5]

A number of studies have proved that microorganisms are present in the bile of the gallbladder, especially in patients of long-term calculous cholecystitis. *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae*, and *Enterococcus faecalis* are the most common bacteria found in bile cultures. These organisms have also been involved in the pathogenesis of gallstones due to enzymatic activity like the presence of β-glucuronidase in addition to the postoperative infectious complications such as surgical site infections, intra-abdominal abscesses, and septicemia.[6-8]

The increased clinical significance of bile microbiology has been further enhanced by the increasing burden of antimicrobial resistance in biliary pathogens. The application of empirical perioperative administration of antibiotics without the knowledge of specific microbial patterns of institutions could be a contributing factor to inappropriate use of antimicrobials and failure of treatment. Previous investigations have shown significant geographical difference in bile culture positivity and the pattern of antibiotic susceptibility indicating the necessity of a regional data to inform rational antimicrobial prophylaxis and therapeutic decision-making.[9,10]

Although the number of gallstone disease cases in northern India is high, scanty information exists on the biliary microbial colonization and susceptibility to antibiotics in patients with chronic calculus cholecystitis at tertiary care centres in Uttar Pradesh. Therefore, the present study was undertaken to evaluate the prevalence and spectrum of biliary microorganisms and to assess their antibiotic susceptibility patterns among patients undergoing cholecystectomy for chronic calculous cholecystitis at a tertiary care centre.

METHODS

Study design and setting

The Department of General Surgery and the Department of Microbiology at Rama Medical College Hospital and Research Centre in Hapur, Uttar Pradesh, India, collaborated to perform this hospital-based prospective observational research. The research spanned 21 months, beginning in March 2024 and ending in December 2025.

Study population

The study included 87 patients diagnosed with chronic calculous cholecystitis who underwent laparoscopic or open cholecystectomy during the study period.

Inclusion criteria

Inclusion in the trial was contingent upon patients meeting the following criteria; patients aged ≥18 years diagnosed with chronic calculous cholecystitis based on clinical examination and ultrasonographic findings, patients undergoing elective laparoscopic or open cholecystectomy, and patients willing to provide written informed consent for participation.

Exclusion criteria

All patients who met the following criteria were not included such as patients diagnosed with acute cholecystitis, empyema gallbladder, gallbladder perforation, or biliary pancreatitis, patients with malignancy of the hepatobiliary system, patients with prior biliary tract surgery or biliary intervention, patients receiving prolonged antibiotic therapy prior to surgery and patients unwilling to participate in the study.

Sample size

The sample size was calculated using the single population proportion formula based on the expected prevalence of biliary microbial colonization among patients with chronic calculous cholecystitis reported in previous literature.

A formula was used to estimate the sample size.

$$N = (Z^2pq)/d^2$$

Considering the standard 95% confidence level, and margin of error 5% and prevalence of biliary microbial colonization among patients with chronic calculous

cholecystitis 6% as reported in previous literature, the minimum required sample size was calculated to be 87 participants.

Sampling method

Consecutive sampling was employed, wherein all eligible patients fulfilling the inclusion criteria during the study period were recruited until the desired sample size was achieved.

Data collection-tools and techniques

Baseline demographic and clinical information, including age, sex, presenting complaints, comorbidities, laboratory investigations, ultrasonographic findings, and operative details, were recorded using a predesigned structured proforma. Surgical route (laparoscopic, open, or converted surgery) and postoperative events, wound infection, were recorded.

Bile sample collection and microbiological analysis

To reduce the possibility of contamination, approximately 2 ml of gallbladder bile aspirate were collected aseptically at the time of cholecystectomy and before the specimen was extracted. Samples that were collected were taken immediately to the Department of Microbiology to be processed. Bile samples were placed on Blood agar and MacConkey agar, and incubated under normal laboratory conditions. Colony morphology, Gram staining and traditional biochemical techniques were used to identify the microbes. Analysis was done with only aerobic bacterial isolates. KirbyBauer disc diffusion test was used to perform antibiotic susceptibility testing based on Clinical and Laboratory Standards Institute (CLSI) guidelines. The sensitivity of the isolated organisms to various antibiotics were noted down and used to determine sensitive, intermediate or resistant based on conventional criteria.

Outcome measures

The primary outcomes of the study were commonness of biliary microbial colonization in patients who have chronic calculous cholecystitis, and spectrum of microorganisms in gallbladder bile. Secondary outcomes included patterns of antibiotic susceptibility of isolated microorganisms and correlation between postoperative wound infection and bile culture positivity.

Statistical analysis

Data was entered into Excel and analyzed using statistical package for the social sciences (SPSS) version 22.0 (IBM Corp., Armonk, NY, USA), a statistical package for the social sciences. For continuous data, we utilized the mean (SD), whereas for categorical variables, we used the frequency and percentage. When applicable, the Chi-square test or Fisher's exact test was used to evaluate associations between categorical variables. Statistical significance was determined by a $p<0.05$.

Ethical considerations

The Rama Medical College Hospital and Research Centre's Institutional Ethics Committee in Hapur, Uttar Pradesh, gave its stamp of approval to the research before it even began. The research ensured that all participants gave written informed permission and that all patient information was kept secret.

RESULTS

A total of 87 patients diagnosed with chronic calculous cholecystitis undergoing cholecystectomy were included in the study. The age of the study population was relatively concentrated in the middle age group with most of the cases being female patients.

Out of the entire number of patients, 70 (80.4) underwent laparoscopic cholecystectomy, 12 (13.8) underwent open cholecystectomy and 5 (5.8) had to be converted to open surgery because of intraoperative complications.

Bile culture positivity

In 20 patients (23%), bile cultures were positive, and 67 patients (77%) provided sterile bile cultures (Table 1). Therefore, about a quarter of patients with chronic calculous cholecystitis had biliary microbial colonization.

Table 1: Participants of the study (n=87) with positive bile culture.

Bile culture result	Frequency (N)	Percentage (%)
Positive	20	23.0
Negative	67	77.0
Total	87	100.0

Distribution of microorganisms isolated from bile aspirates

Of the 20 culture-positive samples, *Escherichia coli* was the most common microorganism, with 45 percent of the isolates being positive, then *Klebsiella pneumoniae* (25 percent). *Enterococcus faecalis* and *Pseudomonas aeruginosa* were both 15% of isolates (Table 2).

Antibiotic susceptibility pattern of isolated microorganisms

Antibiotic susceptibility testing demonstrated organism-specific variability in sensitivity patterns. *Escherichia coli* isolates exhibited relatively higher sensitivity to cefuroxime (66%) and ciprofloxacin (55%), while reduced susceptibility to third-generation cephalosporins was observed.

Klebsiella pneumoniae showed moderate sensitivity to piperacillin–tazobactam (60%) and meropenem (70%).

Table 2: Distribution of microorganisms isolated from bile aspirates (n=20).

Organism isolated	Frequency (N)	Percentage (%)
Escherichia coli	9	45.0
Klebsiella pneumoniae	5	25.0
Enterococcus faecalis	3	15.0
Pseudomonas aeruginosa	3	15.0
Total	20	100.0

All isolates of *Enterococcus faecalis* demonstrated resistance to β-lactam antibiotics; however, complete sensitivity to vancomycin and linezolid was observed. *Pseudomonas aeruginosa* showed greater sensitivity to amikacin (75%) and piperacillin–tazobactam (60%), with comparatively lower susceptibility to ciprofloxacin (Table 3).

Association between bile culture positivity and postoperative wound infection

Postoperative wound infection was observed more frequently among patients with positive bile cultures than among culture-negative patients. Wound infection occurred in 2 of 20 (10%) culture-positive patients compared to 2 of 67 (3%) culture-negative patients. However, statistical analysis revealed that this association was not statistically significant (p>0.05) (Table 4).

Table 3: Antibiotic susceptibility pattern of isolated microorganisms.

Organism	Antibiotics showing greater sensitivity	Sensitivity (%)
Escherichia coli	Cefuroxime	66
	Ciprofloxacin	55
Klebsiella pneumoniae	Piperacillin–tazobactam	60
	Meropenem	70
Enterococcus faecalis	Vancomycin	100
	Linezolid	100
Pseudomonas aeruginosa	Amikacin	75
	Piperacillin–tazobactam	60

Surgical approach

Among the surgical procedures performed, laparoscopic cholecystectomy was the predominant approach (80.4%), followed by open cholecystectomy (13.8%). Conversion from laparoscopic to open surgery was necessary in 5.8% of cases due to operative difficulties (Table 5).

Table 4: Association between bile culture positivity and postoperative wound infection.

Bile culture status	Wound infection present, N (%)	Wound infection absent, N (%)	Total	P value
Culture positive	2 (10.0)	18 (90.0)	20	0.22
Culture negative	2 (3.0)	65 (97.0)	67	
Total	4	83	87	

Table 5: Distribution according to type of surgery (n=87).

Type of surgery	Frequency (N)	Percentage (%)
Laparoscopic cholecystectomy	70	80.4
Open cholecystectomy	12	13.8
Converted to open	5	5.8
Total	87	100.0

DISCUSSION

The present prospective observational study evaluated biliary microbial colonization and antibiotic susceptibility patterns among patients undergoing cholecystectomy for chronic calculous cholecystitis at a tertiary care centre in Uttar Pradesh. The findings demonstrated that 23% of patients exhibited positive bile cultures, with *Escherichia coli* being the predominant isolate, followed by *Klebsiella pneumoniae*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa*. Furthermore, notable variability in antibiotic susceptibility patterns was observed, emphasizing the importance of institution-specific antibiograms for guiding perioperative antimicrobial therapy.

In the present study, 23% of patients demonstrated positive bile cultures, indicating that nearly one-fourth of patients with chronic calculous cholecystitis had biliary microbial colonization despite the absence of overt biliary sepsis. This finding is comparable to that reported by Parekh et al, who observed a culture positivity rate of 24.3% among patients with gallstone disease, suggesting a similar microbiological profile in comparable clinical settings.[4] However, Ballal et al reported a substantially higher positivity rate of 60.9%, which may be attributable to differences in patient demographics, disease severity, perioperative antibiotic exposure, and microbiological processing techniques.[6] Similarly, Mahafzah and Daradkeh documented bile culture positivity of approximately 20% among patients undergoing laparoscopic cholecystectomy, further supporting the observation that biliary colonization is relatively common even in uncomplicated chronic disease.[7]

The predominance of *Escherichia coli* (45%) in the present study is consistent with previously published literature identifying enteric Gram-negative bacilli as the most frequent biliary pathogens in gallstone disease. Similar observations have been reported by Ohdan et al, Parekh et al, and Ballal et al, all of whom demonstrated *E. coli* as the predominant organism isolated from bile cultures.[4,6,8]

The predominance of *E. coli* may be explained by ascending bacterial migration from the duodenum facilitated by gallstone-induced bile stasis and impaired gallbladder emptying. In addition, bacterial enzymes such as β-glucuronidase may contribute to pigment stone formation through bilirubin deconjugation and crystal precipitation, thereby perpetuating chronic inflammation and bacterial persistence within the biliary system.[5]

Apart from *E. coli*, *Klebsiella pneumoniae* (25%), *Enterococcus faecalis* (15%), and *Pseudomonas aeruginosa* (15%) were isolated in the present study. Similar microbial profiles have been reported in both Indian and international literature. Abeysuriya et al documented the presence of *Enterococcus* species and *Pseudomonas* among frequently isolated organisms in bile cultures, whereas Mahafzah and Daradkeh reported predominance of Gram-negative aerobic organisms with occasional Gram-positive isolates.[7,9] The isolation of these organisms suggests polymicrobial biliary colonization and highlights the potential role of intestinal microbial translocation in the pathogenesis of chronic gallbladder inflammation.

An important observation in the present study was the variability in antibiotic susceptibility patterns among biliary isolates. *Escherichia coli* demonstrated relatively greater sensitivity to cefuroxime and ciprofloxacin, whereas diminished susceptibility to third-generation cephalosporins was observed. Similar trends of increasing cephalosporin resistance among Gram-negative biliary pathogens have also been described in previous studies.[6] *Klebsiella pneumoniae* demonstrated moderate susceptibility to piperacillin–tazobactam and meropenem, indicating the retained efficacy of broader-spectrum agents in selected patients.

Notably, all isolates of *Enterococcus faecalis* demonstrated resistance to β-lactam antibiotics but retained complete sensitivity to vancomycin and linezolid, findings that are comparable with previously published microbiological reports.[9] Likewise, *Pseudomonas aeruginosa* exhibited higher sensitivity to amikacin and piperacillin–tazobactam, although lower susceptibility to ciprofloxacin was observed. These findings collectively underscore the growing challenge of antimicrobial resistance and highlight the importance of evidence-based antibiotic selection rather than routine empirical use of broad-spectrum agents.

Although postoperative wound infection occurred more frequently among culture-positive patients (10%) compared to culture-negative patients (3%), the association did not achieve statistical significance ($p>0.05$). This observation can be explained by the fact that the postoperative infectious events and cases of culture positivity were relatively low and limited and were incorporated in the study.

However, the trend observed indicates that biliary microbial colonization can be a possible cause of postoperative infectious morbidity especially in the elderly, diabetic or immunocompromised patients. The same observations have been made in earlier studies assessing postoperative infectious outcomes in patients that underwent cholecystectomy.[10]

The results of the current research have significant clinical implications. Given the apparent microbial colonization and heterogeneity in the patterns of antibiotic susceptibility, the standard practice of using broad-spectrum antibiotics in the routine empirical treatment of all patients undergoing cholecystectomy might not be always justified. Microbiological surveillance and antimicrobial grams that are institution-specific can help select antimicrobial more rationally during perioperative care, minimize unnecessary exposures to antimicrobials, and help in antimicrobial stewardship. Routine bile culture can especially be of benefit to the selected high-risk patients, such as patients with diabetes mellitus, frequent biliary symptoms, long history of the disease, and those that have undergone biliary interventions before.

The current research has a number of strengths. It utilized a prospective study design, standardized intraoperative bile that was collected and microbiological analysis through standard culture and susceptibility testing according to CLSI standards. In addition, the study provides valuable regional microbiological information in North India and the modern evidence of biliary microbial colonization is still sparse.

Nevertheless, there are some limitations that should be considered. To begin with, the research was carried out in one tertiary care facility with a fairly small sample, which might reduce the applicability of results. Second, aerobic organisms were only evaluated, with no assessment of anaerobic bacteria and fungal isolates. Third, no sophisticated molecular diagnostic assays were used like polymerase chain reaction (PCR) which could have resulted in higher rates of microbial detection. Therefore, larger multicentric studies incorporating molecular microbiological methods and long-term postoperative outcomes are warranted to better elucidate the clinical significance of biliary microbial colonization in chronic calculous cholecystitis.

CONCLUSION

The present study demonstrated that biliary microbial colonization was present in approximately one-fourth of patients with chronic calculous cholecystitis undergoing

cholecystectomy, with *Escherichia coli* being the predominant organism isolated from bile cultures. Other microorganisms which were commonly identified were *Klebsiella pneumoniae*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*. Very high variability in patterns of antibiotic susceptibility was observed across biliary isolates which highlights the significance of institution specific antibiograms in informing the selection of antimicrobials used in the perioperative period.

Even though postoperative wound infection was more common in culture-positive patients, there was no statistically significant correlation. The results indicate that routine bile culture can be helpful in a small group of high-risk patients and can be used to help improve evidence-based antimicrobial stewardship. They suggest larger multicentric studies that would include anaerobic cultures and molecular diagnostic to further clarify the clinical implications of biliary microbial colonization in chronic calculous cholecystitis.

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