Original Article

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Microbial Flora Dynamics in Gallstones: A Prospective Observational Study of Cholecystectomy Patients

Microbiology Section

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ABSTRACT

Introduction: Gallstone disease is a common cause of morbidity worldwide, with numerous studies suggesting the role of bacteria in the pathogenesis of gallstone formation and in infective complications arising from stones lost in the peritoneal cavity post-surgery. Although molecular and microscopy methods can detect the presence of bacteria, they do not provide information about the infectious potential of the microorganisms.

Aim: To analyse the bacteriological profile of gallstones in patients with cholelithiasis undergoing open or laparoscopic cholecystectomy.

Materials and Methods: A prospective observational study was conducted on patients undergoing cholecystectomy for symptomatic cholelithiasis from February 2024 to May 2025 in the Departments of Surgery and Microbiology at a tertiary care hospital in Central Maharashtra (Chhatrapati Sambhajinagar), India. The study included 76 patients undergoing open or laparoscopic cholecystectomy for acute, chronic, or acute-on-chronic cholecystitis. Demographic details like age and gender were recorded, along with clinical presentation, intraoperative findings, and co-morbidities. Post-cholecystectomy, gallstones were aseptically collected and subjected to culture and sensitivity testing. Positive cultures were further processed for identification and susceptibility using the Vitek 2 system, while negative cultures were reported after five days of incubation.

Results: The peak incidence in females was observed in the 41-60 years age group (20 cases, 26.3%), whereas in males it was observed in the 61-80 years age group (23 cases, 30.3%). Culture positivity was observed in 49 gallstones (64.5%). Enteric bacteria predominated amongst the isolates, with *Escherichia coli* (*E. coli*) being the most common (18 cases, 31.57%), followed by *Enterococcus species* (8 cases, 14.03%). *Salmonella* paratyphi A was isolated in 2 cases (3.50%). *Escherichia coli* showed high susceptibility to aminoglycosides (18 cases, 100%), tigecycline (17 cases, 94.4%), and carbapenems (16 cases, 88.8%). Enterococci were highly susceptible to teicoplanin, tigecycline and linezolid. Follow-up was conducted post-discharge at 5 days, 15 days, and monthly thereafter.

Conclusion: Gallstones act as a significant microenvironment for bacterial colonisation. Gallstone cultures, rather than bile cultures alone, provide a better understanding of gallstone-associated microbiota, their role in disease pathogenesis, and postoperative complications. This information can help optimise antibiotic strategies, especially in high-risk or polymicrobial cases, leading to improved patient outcomes.

Keywords: Antibiotics in cholecystitis, Antimicrobial stewardship, Gallstone culture

INTRODUCTION

Cholelithiasis is the most common biliary pathology globally and represents a significant cause of morbidity that often necessitates cholecystectomy. Gallstone disease is a chronic disorder of the hepatobiliary system, arising from impaired metabolism of cholesterol, bilirubin, and bile acids, which clinically manifests as the formation of stones in the hepatic bile duct, common bile duct, or gallbladder. The prevalence of cholelithiasis in India ranges from 2% to 29% and has been increasing in recent years [1]. Gallstones are typically categorised into cholesterol, pigment, or mixed types based on their composition [2]. While metabolic, genetic, and environmental factors contribute to gallstone pathogenesis, research indicates that bacteria may also play a role, particularly in pigment stones [3]. Various concepts exist regarding the role of bacteria in gallstone formation and associated complications [4-6]. Live bacteria have been reported within gallstones, with both enteric and non enteric organisms capable of surviving in the stone matrix [5,7]. Gallstones themselves provide a niche for bacterial colonisation, which explains the presence of non enteric bacteria [3]. Studies using gallstone culture, molecular genetics, and scanning electron microscopy have demonstrated the presence of bacteria within gallstones. However, bacterial culture of the stone nidus remains the gold standard for studying the pathogenesis and infectious potential of gallstones [7,8].

Most prior studies have focused on bile cultures rather than culturing the stone nidus itself [9,10]. This study is novel because it specifically cultures the nidus of gallstones to isolate microorganisms. The presence of live microorganisms within the nidus can significantly impact patient outcomes if stones are spilled into the peritoneal cavity during surgery. Therefore, culturing these microorganisms and studying their antimicrobial susceptibility patterns can guide postoperative management and antimicrobial stewardship. The current study aimed to analyse the bacteriological profile of gallstones in patients undergoing open or laparoscopic cholecystectomy.

MATERIALS AND METHODS

This prospective observational study was conducted from February 2024 to May 2025 in the Department of Surgery, in collaboration with the Department of Microbiology, at a tertiary care hospital, Dr. Hedgewar Rugnalaya, Chhatrapati Sambhajinagar, Maharashtra, India. Ethics committee approval was obtained (EC/Approval/06/2023/02). Registration number of the Ethics Committee: ECR/641/Inst/MH/2014-RR20. This was a time-bound study, and all 76 subjects available during the study period were taken into consideration.

Inclusion criteria: Patients from Central Maharashtra undergoing open or laparoscopic cholecystectomy for cholelithiasis with acute, chronic, or acute-on-chronic cholecystitis were included in the study.

Exclusion criteria: Patients managed conservatively were excluded from the study. These patients were admitted, kept nil by mouth (NBM), given intravenous fluids, analgesics, and empirical antibiotic therapy (Cefuroxime).

Study Procedure

All eligible patients meeting the above criteria and undergoing cholecystectomy were enrolled using consecutive sampling. Patients attending surgical clinics with chief complaints of right hypochondriac pain, nausea, and fever were thoroughly examined after detailed history taking. Demographic details like age, gender, and locality were recorded. Investigations, including complete blood count, liver function tests, kidney function tests, and abdominal ultrasonography, were performed and documented. Diagnosis was confirmed by ultrasonography, contrast-enhanced computed tomography, or magnetic resonance cholangiopancreatography, as required.

After cholecystectomy, the gallbladder specimen was opened in the operating theatre. Stones were transferred to a sterile container containing sterile normal saline and transported to the Microbiology laboratory. One stone was selected, cleaned using sterile normal saline and 70% ethanol for 10 minutes to ensure surface disinfection [7]. The core was processed using sterile saline in an autoclaved mortar and pestle and inoculated into a BacT/Alert bottle, which was incubated in the BacT/Alert system. After a positive flag alert, the respective bottle was removed, and subcultures were performed on Blood agar and MacConkey agar, followed by overnight incubation under aerobic conditions. Anaerobic cultures were not performed. Colony morphology was examined after subculture, and if more than one colony type was observed, each was processed further after purification and Gram staining. Identification and susceptibility testing were performed using the Vitek 2 system. Antimicrobial susceptibility was reported according to Clinical and Laboratory Standards Institute (CLSI) guidelines based on the breakpoint Minimum Inhibitory Concentration (MIC) system [11]. Resistance mechanisms in gram-negative bacilli, including Extended-Spectrum Beta-Lactamases (ESBL) and carbapenemase phenotypes, were detected using the Vitek 2 system. Negative cultures were reported after five days of incubation. Follow-up was conducted post-discharge at 5 days, 15 days, and monthly. Patients were examined for wound healing and evidence of postoperative wound infection.

STATISTICAL ANALYSIS

Results were analysed using MS Excel and WHONET 5.6 software.

RESULTS

The peak incidence in females was observed in the 41-60 years age group (20 cases, 26.3%), and in males, it was observed in the 61-80 years age group (23 cases, 30.3%) [Table/Fig-1].

Age group (years)	Female	Male	Total
20-40	5 (6.6)	6 (7.9)	11 (14.5)
41-60	20 (26.3)	13 (17.1)	33 (43.4)
61-80	8 (10.5)	23 (30.3)	31 (40.8)
80-100	0	01 (1.3)	01 (1.3)

[Table/Fig-1]: Age and gender distribution.

Of the 76 patients with cholelithiasis, 41 (54%) underwent cholecystectomy for chronic cholecystitis, 19 (25%) for acute cholecystitis, and 13 (17.1%) for acute-on-chronic cholecystitis. The remaining three patients included one (1.31%) with gallbladder carcinoma and two (2.6%) with complications from slipped gallstones post-cholecystectomy.

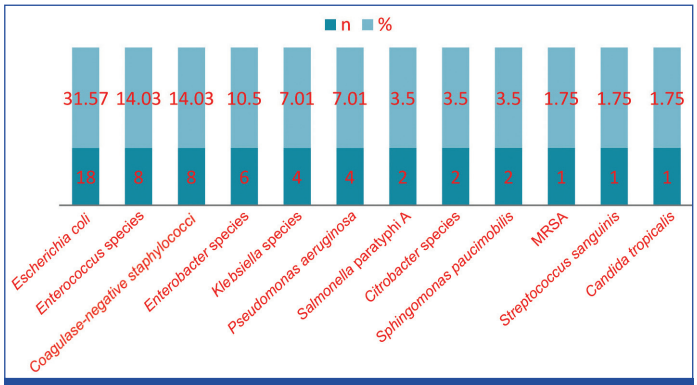
Co-morbidities were present in 71 of 76 patients (93.4%). Among males (52 patients, 73.2%), the most common co-morbidities were hypertension (23, 44.2%), diabetes mellitus (16, 30.8%), and ischaemic heart disease (6, 11.5%). Among females (19 patients, 26.8%), hypertension (7, 36.8%) and diabetes mellitus (6, 31.5%) were most common [Table/Fig-2].

Co-morbidity	Male n (%) N=52	Female n (%) N=19
DM	16 (30.8)	6 (31.5)
IHD	6 (11.5)	2 (10.9)
HTN	23 (44.2)	7 (36.8)
COPD	4 (7.6)	1 (5)
Epilepsy	1 (1.9)	_
HS	1 (1.9)	_
Hypothyroidism	_	2 (10.9)
BPH	1 (1.9)	_
Obesity, AKI	_	1 (5)

[Table/Fig-2]: Distribution of patients as per the associated co-morbidities.
HS: Hereditary spherocytosis; BPH: Benign prostatic hypertrophy; IHD: Ischaemic heart disease; DM: Diabetes mellitus; COPD: Chronic obstructive pulmonary disease; HTN: Hypertension; AKI: Acute kidney injury

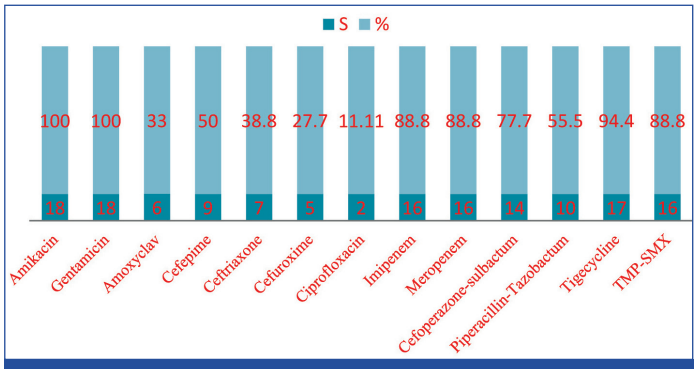
Laparoscopic cholecystectomy was performed in 36 patients (47%), and open cholecystectomy in 40 patients (53%). Among the 76 gallstones studied, culture positivity was observed in 49 (64.47%). A total of 57 isolates were recovered, including 38 (66.7%) Gram-Negative Bacilli (GNB), 18 (31.8%) Gram-Positive Cocci (GPC), and one (1.7%) *Candida* species. Polymicrobial growth was observed in six cases (7.9%).

Enteric bacteria predominated amongst the isolates. The most commonly isolated organism was *Escherichia coli* (18 cases, 31.57%), followed by *Enterococcus* species (8 cases, 14.03%). *Salmonella* paratyphi A was isolated in two cases (3.50%) [Table/Fig-3].



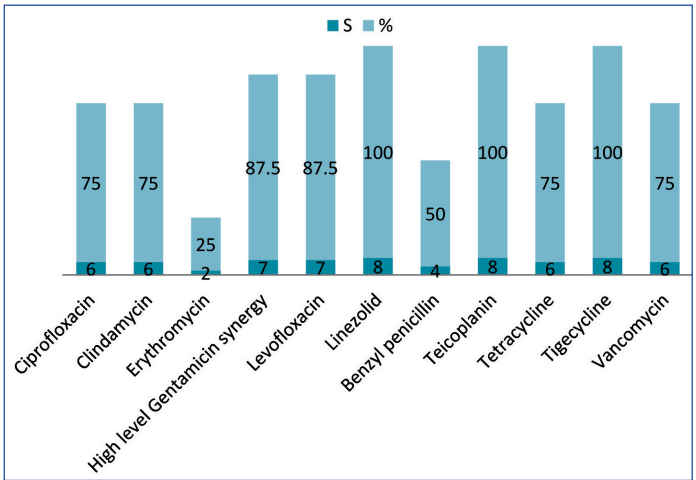
[Table/Fig-3]: Microbiological analysis of gallstones (n=57).

Antimicrobial susceptibility patterns were documented descriptively to show the distribution of susceptibility among bacterial isolates from gallstones and were not intended to generate a hospital-level antibiogram. [Table/Fig-4,5] depict the antimicrobial susceptibility of *Escherichia coli* and *Enterococcus* species.



[Table/Fig-4]: Antimicrobial susceptibility pattern of *Escherichia coli* (n=18).

Clinical outcomes are summarised in [Table/Fig-6]. Outcomes were determined based on patient presentation, type of surgery,



[Table/Fig-5]: Antimicrobial susceptibility pattern of *Enterococcus* species (n=8).

Outcome	n (%)
Uneventful recovery	68 (89.47)
Lengthened hospital stay	4 (5.26)
Mortality	1 (1.31)
Postoperative wound infection	1 (1.31)
Biliary leak	1 (1.31)
Postoperative haematoma	1 (1.31)

[Table/Fig-6]: Clinical outcomes.

intraoperative findings, culture and susceptibility results, and postoperative haemodynamics.

Postoperative complications included one patient with a biliary leak due to a slipped stone, managed successfully with Endoscopic Retrograde Cholangiopancreatography (ERCP). One patient required re-exploration for an intra-abdominal haematoma. Mortality occurred in one high-risk patient undergoing cholecystectomy due to acute coronary syndrome; this patient had a history of hypertension and prior stroke. Gallstone culture in this case was sterile. Postoperative wound infection was observed in one patient, with *Escherichia coli* and *Enterococcus* species isolated from both the gallstone and wound discharge. This patient had multiple co-morbidities and underwent ERCP for choledocholithiasis.

Sepsis occurred in four patients during the course of acute gangrenous cholecystitis, requiring intensive care due to persistent tachycardia, fever, and haemodynamic instability.

On average, the remaining patients required a hospital stay of four to seven days. Follow-up was conducted at five days, 15 days (seven patients did not visit the surgery clinic at this time point), and one month, as depicted in [Table/Fig-7].

Time point	Patients followed	Assessment done	Results
5 days	75 (98.6%)	Fever, formation of seroma, haematoma, discharge from the surgical site	1 patient developed a surgical site infection
15 days	68 (89.4%)	Wound healing, resolution of symptoms	Satisfactory recovery in all patients, including patients who had lengthened stay due to haematoma formation and development of sepsis.
1 month	75 (98.6%)	Recovery status, diet tolerance, and biliary leak	1 Re-admission due to biliary leak The rest all recovered.

[Table/Fig-7]: Details of follow-up of the patients.

DISCUSSION

The dynamics between gallstone-associated bacteria and infection have been extensively reviewed in the literature. Enteric bacteria ascend from the gastrointestinal tract into the biliary system and produce enzymes like beta-glucuronidase and phospholipase, leading to precipitation of bilirubin and calcium salts and subsequent pigment stone formation [3,12].

In the present study, 41 patients (53.9%) presented with chronic cholecystitis, 13 (17.1%) with acute-on-chronic cholecystitis, and 19 (25%) with acute cholecystitis. One patient (1.3%) had gallbladder carcinoma, and two cases (2.6%) presented with slipped gallstones post-cholecystectomy. Intraoperative findings included thickened gallbladder walls, adhesions, gallbladder perforation, gangrenous changes, and empyema. Laparoscopic cholecystectomy was performed in 36 patients (47%), while 40 patients (53%) underwent open procedures. In comparison, the study by Shiekh MR et al., reported that 54.76% of patients had thickened gallbladder walls, suggesting chronic inflammation, and Gharaibeh KIA et al., observed gallbladder perforation in 31% of acute cholecystitis cases, demonstrating that clinical diagnosis correlated with intraoperative findings [13,14].

In the current study, 49 gallstones (64.47%) were culture-positive. Enteric bacteria (38 isolates, 66.7%) were the predominant microorganisms, with *Escherichia coli* being the most common (18 isolates, 31.57%). Hazrah P et al., reported 81% culture positivity in cholelithiasis cases [7], while Soman KC et al., found enteric bacteria in 29.54% of isolates [15]. Hazrah P et al., also identified *Klebsiella* species as the most frequently isolated organism (18%) [7]. *Streptococcus sanguinis*, a rare pathogen, was isolated in the present study; Soman KC et al., have also reported *Streptococcus* species from gallstones [15]. *Salmonella* paratyphi A, isolated in two cases (3.50%) in this study, may indicate subclinical biliary carriage in endemic areas. Over 80% of chronic *Salmonella* carriers have gallstones, a significantly higher prevalence than the general population [16,17]. Polymicrobial growth was observed in six cases (7.89%), primarily in patients with co-morbidities such as diabetes mellitus and acute-on-chronic cholecystitis. Katyal A et al., reported polymicrobial infections in 11.43% of cases, associated with chronic inflammation, co-morbidities, or biliary tract interventions [9].

In the present study, *Escherichia coli* exhibited high susceptibility to aminoglycosides (18/18, 100%), tigecycline (17/18, 94.4%), carbapenems (16/18, 88.8%), and trimethoprim-sulfamethoxazole (16/18, 88.8%). Similar findings have been reported by Huang X-M et al., with 100% susceptibility to carbapenems [18], Gomes PRL et al., with 85% susceptibility to aminoglycosides [19], and Qassem M et al., with 100% susceptibility to tigecycline [20]. Fourteen isolates (45%) were ESBL producers in this study, consistent with literature reports by Huang XM et al., [18]. Cefoperazone-sulbactam (23/31, 74.2%) and piperacillin-tazobactam (15/27, 56.3%) showed better susceptibility rates for ESBL-producing enterobacterales. Carbapenem-resistant Enterobacterales (CRE) were observed in three cases (13%), but comparative studies are limited.

Enterococcus species accounted for eight isolates (14.03%) in this study. They exhibited 100% susceptibility to teicoplanin, tigecycline, and linezolid; 75% susceptibility to quinolones and high-level gentamicin; and 62.5% susceptibility to both vancomycin and penicillin. Two isolates of *Enterococcus faecium* were vancomycin-resistant. Qassem M et al., reported 100% susceptibility of *Enterococcus* species to tigecycline [20]. Other studies have shown most *Enterococcus* species susceptible to vancomycin and penicillin, except *Enterococcus faecium* [18,21]. Coagulase-negative staphylococci (CONS) isolates (8/57, 14.03%) were associated with co-morbidities and showed 100% susceptibility to glycopeptides, tigecycline, and linezolid. The literature highlights the pathogenic role of CONS in biofilm formation [6,22], with similar findings reported by Bora P et al., [23].

All non fermenting gram-negative bacilli in this study were 100% susceptible to aminoglycosides, cephalosporins, beta-lactam/beta-lactamase inhibitor (BL-BLI) combinations, carbapenems, and trimethoprim-sulfamethoxazole, with moderate susceptibility to quinolones (66-83%). Farooq MO et al., reported a similar susceptibility pattern [24]. High susceptibility may also reflect the

absence of prior antimicrobial therapy in these patients, in contrast to increasing resistance observed in hospital environments.

In this study, the combination of cholecystectomy and second/third-generation cephalosporins or BL-BLI therapy led to favourable clinical outcomes. In some chronic cholecystitis cases, clinical improvement was observed despite in-vitro resistance. In 13 cases, the same microorganism was isolated from both the gallstone and the pus or peritoneal fluid of the patient. Microbiology culture reports guided the escalation or de-escalation of antibiotics due to close collaboration between the Microbiology and Surgery departments, supporting antimicrobial stewardship. The current study provides a comprehensive profile of gallstone-associated bacteria.

Limitation(s)

A limitation of this study was the absence of anaerobic culture, which could not be performed due to constraints related to establishing an anaerobic setup and limited financial feasibility.

CONCLUSION(S)

Culture positivity was observed in the majority of gallstones, supporting their role as a reservoir of viable bacteria. The most commonly isolated bacteria were *Escherichia coli*, followed by *Enterococcus* species. The predominance of enteric bacteria suggests gastrointestinal stasis and translocation of gut flora into the biliary tract, contributing to gallstone formation. Identical isolates from gallstones and peritoneal fluid or pus highlights the clinical value of gallstone culture in guiding appropriate antimicrobial treatment. Treatment of biliary infections should consider ESBL-producing bacteria. Targeted therapy based on gallstone culture enhances patient outcomes and supports antimicrobial stewardship.

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