

BILE CULTURE ISOLATES, ANTIMICROBIAL SUSCEPTIBILITY AND CLINICAL PROFILE IN A TERTIARY CARE HOSPITAL IN SOUTH INDIA: A RETROSPECTIVE STUDY

Laxmi Narayana B¹ and Roshni R. Rao²

¹Department of Medical Laboratory Sciences, Yenepoya School of Allied Health Sciences, Yenepoya (deemed to be) University, Mangalore, Karnataka, India

²Department of Microbiology, Medical Laboratory Technology, Indiana Institute of Allied Health Sciences, Mangalore, Karnataka, India

*Corresponding author: Laxmi Narayana B, E-mail: laxminarayanab@yenepoya.edu.in, ORCID iD: <https://orcid.org/0009-0005-1954-7211>, E-mail of co-author: Roshni R. Rao, roshnirao017@gmail.com

ABSTRACT

Biliary tract infections are a frequent cause of sepsis in patients with biliary obstruction, and empirical therapy depends on local knowledge of the causative organisms and their resistance. This retrospective study describes the microbial isolates recovered from bile, their antimicrobial susceptibility, and the associated predisposing factors and complications in a tertiary care hospital in Mangalore, South India. All bile samples received from August 2015 to July 2016 were reviewed. Isolates were identified by conventional biochemical tests, and susceptibility was determined by disk diffusion. Of 161 bile samples, 108 (67.1%) showed significant growth. *Escherichia coli* was the most frequent isolate (30.9%), followed by *Pseudomonas* spp. (23.7%), *Klebsiella* spp. (17.3%) and *Enterococcus* spp. (9.4%). Susceptibility to cefotaxime was 4.7% in *E. coli* and 16.7% in *Klebsiella* spp., and extended-spectrum beta-lactamase producers of both organisms were detected. Imipenem and amikacin were the most active agents against *E. coli* (81.4% each) and *Pseudomonas* spp. (78.1% and 87.5%), and imipenem and cefoperazone/sulbactam against *Klebsiella* spp. (87.5% and 83.3%). However, 39.5% of *E. coli* and 33.3% of *Klebsiella* isolates were not susceptible to meropenem. All *Enterococcus* isolates were susceptible to vancomycin and teicoplanin. Among 75 patients with complete records, cholangitis was the most common diagnosis (50.7%), and cholelithiasis (45.3%), carcinoma (30.7%) and biliary stenting (29.3%) were the main predisposing factors; sepsis occurred in 4.0% and multiple organ dysfunction in 2.7%. Gram-negative bacilli with high resistance to third-generation cephalosporins predominated, and reduced carbapenem susceptibility was already evident. Routine bile culture with susceptibility testing, interpreted against current breakpoints, and continued local surveillance are needed to guide empirical therapy and antimicrobial stewardship.

KEYWORDS: Bile culture; Biliary tract infection; Antimicrobial susceptibility; *Escherichia coli*; Extended-spectrum beta-lactamase; Cholangitis

INTRODUCTION

Biliary tract diseases are among the most common disorders encountered in clinical practice and include cholecystitis, acute cholangitis, choledocholithiasis, biliary obstruction and hepatobiliary malignancies. Bile is sterile under normal conditions. Continuous antegrade bile flow, the sphincter of Oddi, the bacteriostatic action of bile salts and biliary immunoglobulin A together prevent bacteria from ascending from the duodenum. When these defences are breached by gallstones, strictures, tumours, biliary instrumentation or an indwelling stent, bacteria colonize the biliary tree and biliary tract infection may follow (Karpel et al., 2011; Kaya et al., 2012; Zimmer and Lammert, 2015).

The development of infection is primarily associated with biliary obstruction, raised intrabiliary pressure and microbial invasion of the bile ducts. Under obstructive conditions, bacteria proliferate within stagnant bile and may translocate into the bloodstream. If left untreated, the infection may progress to a severe inflammatory response, bacteraemia, sepsis, multiple organ dysfunction and death. Early diagnosis, prompt biliary drainage and timely administration of appropriate antimicrobial therapy are therefore essential to reduce morbidity and mortality (Kaya et al., 2012; Shenoy et al., 2014; Zimmer and Lammert, 2015).

Bile culture remains an important diagnostic tool because it identifies the causative organisms and determines their antimicrobial susceptibility, which allows empirical therapy to be narrowed or changed once results are available. Gram-negative enteric bacteria, especially *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, are the predominant pathogens isolated from infected bile, although Gram-positive bacteria such as *Enterococcus* spp., anaerobes and *Candida* species are also encountered, particularly after instrumentation (Karpel et al., 2011; Salvador et al., 2011; Kaya et al., 2012).

The spectrum of biliary pathogens is not uniform. In a 12-year review from a Korean tertiary centre, *Enterococcus* became the most frequently isolated genus from bile, and vancomycin-resistant enterococci increased over time (Kwon et al., 2013). Resistance among Gram-negative bacilli is a further concern. Extended-spectrum beta-

lactamases (ESBLs) hydrolyse third-generation cephalosporins, which have long been used for biliary infection, and ESBL-producing organisms are frequently resistant to fluoroquinolones and aminoglycosides as well (Paterson and Bonomo, 2005). In Indian series, *E. coli* from bile has shown high resistance to third-generation cephalosporins and ciprofloxacin (Sahu et al., 2011), and ESBL production has been reported in 66% to 73% of biliary *E. coli* and *Klebsiella* isolates (Shenoy et al., 2014).

Recent reports show that the problem has not eased. In a one-year review of 226 bile samples from North India, about 70% of Gram-negative isolates were multidrug-resistant and one-quarter of enterococci were resistant to vancomycin (Gupta et al., 2024). A five-year study of 880 patients with choledocholithiasis in China found ESBL production in 44.1% of *E. coli* and 38.9% of *K. pneumoniae*, together with a rise in Gram-positive cocci (Zhang et al., 2024). A prospective study in two European centres showed that the proportion of multidrug-resistant bile isolates differed three-fold between hospitals in different countries (Cozma et al., 2025). Such differences mean that resistance data cannot be transferred from one setting to another.

For these reasons, current guidelines recommend that empirical antimicrobial therapy for acute cholangitis and cholecystitis be selected according to the severity of illness and the local antibiogram, and that local susceptibility data be monitored and updated regularly (Gomi et al., 2018). Because the microbial profile and resistance patterns vary across regions and healthcare settings, continuous surveillance of bile isolates in each institution is necessary to guide empirical antibiotic selection (Ellis and Chapman, 2010; Kaya et al., 2012). The present study was therefore undertaken to identify the microbial isolates recovered from bile cultures, to describe their antimicrobial susceptibility patterns, and to document the predisposing factors and complications among patients investigated for biliary tract infection in a tertiary care hospital in South India.

MATERIAL AND METHODS

Study design and setting

This was a retrospective, time-bound, descriptive study conducted in the Central Laboratory of Kasturba Medical College Hospital, Ambedkar Circle, Mangalore, Karnataka, India, a tertiary care teaching hospital. The study covered a period of one year, from August 2015 to July 2016. The study was carried out by the authors while they were working at this institution. The report follows the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) recommendations for observational studies (von Elm et al., 2008).

Ethical approval

The study was conducted after clearance from the Institutional Ethics Committee of Kasturba Medical College, Mangalore (reference number IEC KMC MLR 09-16/216). Data were obtained from existing laboratory and hospital records, and no samples were collected for the purpose of the study. The committee waived the requirement for individual informed consent because the study involved only a retrospective review of existing records.

Eligibility criteria

Patients of all ages and both sexes who underwent bile culture for suspected biliary tract infection during the study period were eligible. These included patients with cholelithiasis, cholecystitis, cholangitis, biliary obstruction and other hepatobiliary disorders. All bile culture reports were reviewed irrespective of culture positivity. Culture-positive samples showing significant microbial growth were included in the analysis of microbial profile and antimicrobial susceptibility. Patients with complete demographic and clinical records were included in the analysis of predisposing factors and complications.

Bile specimens documented in the laboratory records as contaminated or improperly collected were excluded, as were patients with incomplete microbiological records. Sterile cultures were excluded from the analysis of isolates and susceptibility patterns but were retained for the calculation of culture positivity.

Sample size and sampling

The study was time-bound, and the sample comprised every eligible bile sample received during the one-year study period (complete enumeration by consecutive sampling), so no sample size was fixed in advance. All bile samples received were screened for eligibility, and 161 samples were included in the microbiological analysis. Complete demographic and clinical records were available for 75 patients, who formed the clinical analysis set (Figure 1).

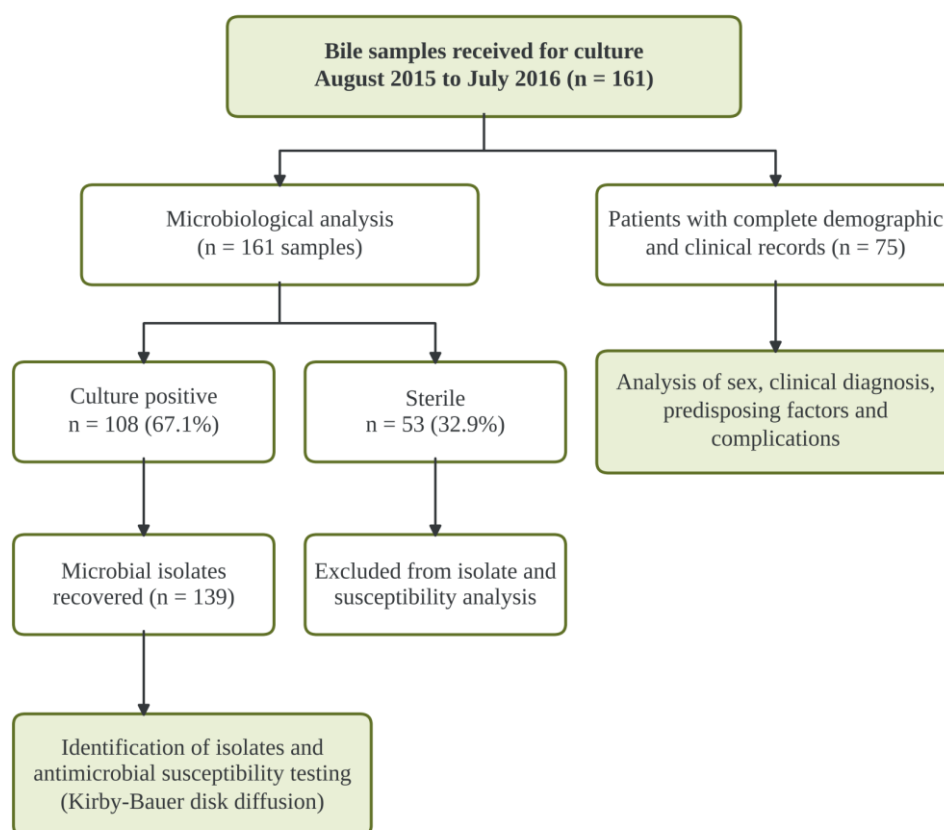


Figure 1. Flow of bile samples and patients through the study. The 75 patients with complete records are drawn from the patients who contributed the 161 samples; the number of distinct patients contributing samples could not be determined from the laboratory summary.

The adequacy of the sample was assessed after data collection. With 161 samples and an observed culture positivity of 67.1%, the 95% confidence interval for the primary outcome has a half-width of 7.3 percentage points (single-proportion formula, $n = Z^2 p(1 - p)/d^2$ (Charan and Biswas, 2013)), which was considered adequate for a descriptive estimate.

Bile sample collection

Bile had been collected aseptically from patients with suspected biliary tract infection during endoscopic retrograde cholangiopancreatography (ERCP) or at laparotomy, from the common bile duct or the gallbladder. About 2 to 3 mL of bile was collected in a sterile syringe or container, and cultures were set up within 1 hour of collection.

Culture and identification

Samples were inoculated onto 5% sheep blood agar and MacConkey agar and into thioglycollate broth, and incubated at 37°C for 24 hours. Bacteria isolated aerobically were identified by conventional methods, namely Gram stain, motility, catalase, oxidase, indole, citrate utilization, urease and triple sugar iron agar reactions, supplemented by other biochemical tests as required. These were the routine methods of the laboratory during the study period and have been described previously (Shenoy et al., 2014).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the Kirby-Bauer disk diffusion method (Bauer et al., 1966) on Mueller-Hinton agar, and the results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines in use at the time; the edition applied was not recorded. Gram-negative isolates were tested against aminoglycosides (amikacin, gentamicin), cephalosporins (cefotaxime, ceftazidime), beta-lactam/beta-lactamase inhibitor combinations (cefoperazone/sulbactam, piperacillin/tazobactam), carbapenems (imipenem, meropenem, ertapenem), ciprofloxacin and cotrimoxazole, according to the organism; not every agent was reported for every organism (Table 1). CLSI does not provide interpretive criteria for cefoperazone/sulbactam, and the criteria applied by the laboratory for this agent were not recorded. *Enterococcus* isolates were tested against penicillin, ampicillin, amoxicillin/clavulanate, piperacillin/tazobactam, high-level gentamicin, high-level streptomycin, vancomycin and teicoplanin; they were not identified to species level. Results for agents to which an organism is intrinsically resistant, namely ertapenem and cotrimoxazole for *Pseudomonas* spp., were not analysed as susceptibility results, in line with expert rules for susceptibility testing (Leclercq et al., 2013). Results recorded for netilmicin and tigecycline could not be verified against the source records and were excluded from the analysis.

Detection of ESBL production

ESBL production in *E. coli* and *Klebsiella* spp. was tested by disk diffusion, comparing ceftazidime (30 µg) with ceftazidime-clavulanic acid (30/10 µg) and cefotaxime (30 µg) with cefotaxime-clavulanic acid (30/10 µg). An increase of 5 mm or more in the zone diameter of either agent tested with clavulanic acid, compared with the zone of the same agent tested alone, was taken as evidence of ESBL production, in accordance with the CLSI phenotypic confirmatory method (Shenoy et al., 2014). Carbapenemase production was not tested.

Quality control

Escherichia coli ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as the negative and positive controls, respectively, for ESBL testing, and *Pseudomonas aeruginosa* ATCC 27853 was used for quality control of susceptibility testing.

Data sources and variables

Data were retrieved from the microbiology laboratory registers and the hospital medical records. The laboratory summary available for this analysis recorded organism frequencies and susceptibility as percentages rather than as counts. The numbers of isolates given in this report were derived from those percentages and the denominators consistent with them (139 isolates in total; 43 *E. coli*, 24 *Klebsiella* spp., 32 *Pseudomonas* spp. and 13 *Enterococcus* spp. with susceptibility results), and percentages were recalculated to one decimal place. The summary did not identify repeat samples from the same patient, so the unit of analysis for the microbiological results is the sample, not the patient. The variables collected were age, sex, clinical diagnosis, predisposing factors (cholelithiasis, biliary stenting, carcinoma and liver cirrhosis), bile culture result, organisms isolated and their antimicrobial susceptibility, and complications such as sepsis and multiple organ dysfunction syndrome (MODS). The primary outcome was bile culture positivity. The secondary outcomes were the frequency of each organism among all isolates, the proportion of isolates of each organism susceptible to each antimicrobial agent, and the frequency of predisposing factors and complications.

Control of bias

To limit selection bias, all consecutive samples received during the study period were included, irrespective of culture result. The microbiological and clinical analyses used separate denominators: all 161 samples for culture positivity, all isolates for the distribution of organisms, the isolates of each organism tested for susceptibility, and the 75 patients with complete records for clinical variables. Missing data were not imputed.

Statistical analysis

The analysis was descriptive. Categorical variables are presented as frequencies and percentages. Culture positivity was calculated as the proportion of samples with significant growth among all samples included. The frequency of each organism is expressed as a percentage of all isolates. Susceptibility is expressed as the proportion of susceptible isolates among the isolates of each organism tested against that agent. Ninety-five percent confidence intervals (CI) for the main proportions were calculated by the Wilson score method, which performs well for small samples and for proportions close to 0 or 1 (Newcombe, 1998). No hypothesis tests were applied, because the study was not designed to compare groups.

RESULTS

Bile culture results

A total of 161 bile samples were included during the study period. Of these, 108 (67.1%; 95% CI 59.5-73.9) showed significant microbial growth and 53 (32.9%) were sterile (Figure 1).

Distribution of isolates

The percentages recorded in the laboratory summary correspond to a total of 139 isolates from the 108 culture-positive samples, indicating that some samples yielded more than one organism. *Escherichia coli* was the most frequent isolate (43; 30.9%; 95% CI 23.9-39.0), followed by *Pseudomonas* spp. (33; 23.7%; 95% CI 17.4-31.5), *Klebsiella* spp. (24; 17.3%; 95% CI 11.9-24.4) and *Enterococcus* spp. (13; 9.4%; 95% CI 5.5-15.3). The remaining isolates were *Enterobacter* spp. (5; 3.6%), *Citrobacter* spp. (4; 2.9%), *Candida* spp. (2; 1.4%), *Streptococcus* spp., *Staphylococcus aureus* and *Proteus* spp. (1 each; 0.7%), and other organisms (3; 2.2%) (Figure 2). The organisms named in the laboratory summary accounted for 130 isolates (93.5%); the remaining nine (6.5%) were not individually specified in the available records.

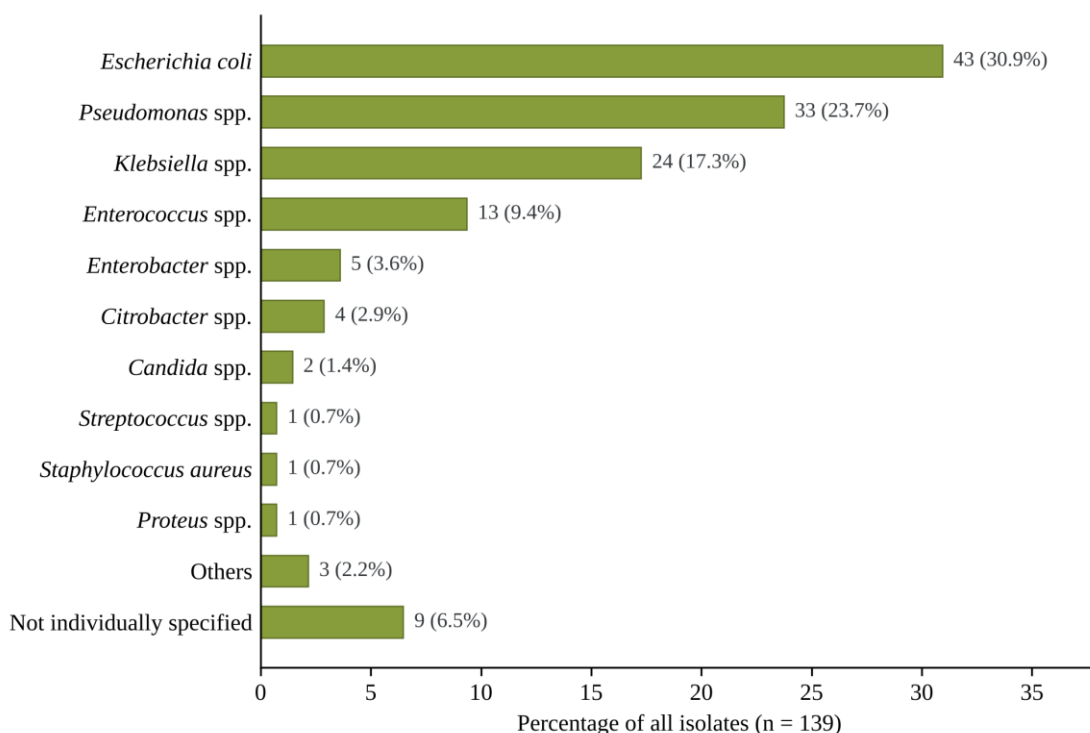


Figure 2. Distribution of microbial isolates recovered from culture-positive bile samples. Values beside the bars are number of isolates (percentage of all isolates). Nine isolates were not individually specified in the available records.

Gram-negative bacilli therefore accounted for 110 isolates (79.1%), of which the Enterobacterales (*E. coli*, *Klebsiella*, *Enterobacter*, *Citrobacter* and *Proteus*) contributed 77 (55.4%) and *Pseudomonas* spp. 33 (23.7%). Gram-positive cocci accounted for 15 isolates (10.8%) and *Candida* spp. for two (1.4%).

ESBL-producing isolates

ESBL production was detected among isolates of *Klebsiella* spp. and *E. coli*. Of the ESBL-producing isolates, 64% were *Klebsiella* spp. and 36% were *E. coli*. The number of isolates tested for ESBL production was not available, so species-specific ESBL rates could not be calculated, and these proportions describe only the composition of the ESBL-positive group.

Antimicrobial susceptibility of Gram-negative isolates

The susceptibility of the three predominant Gram-negative organisms is shown in Table 1 and Figure 3. *E. coli* was most susceptible to amikacin and imipenem (81.4% each), followed by cefoperazone/sulbactam (65.1%), meropenem (60.5%) and ertapenem (58.1%). Susceptibility was lower to gentamicin (37.2%) and cotrimoxazole (23.3%), and lowest to cefotaxime (4.7%).

Table 1. Antimicrobial susceptibility of the predominant Gram-negative isolates from bile.

Antimicrobial agent	<i>Escherichia coli</i> (n = 43)	<i>Klebsiella</i> spp. (n = 24)	<i>Pseudomonas</i> spp. (n = 32)
Amikacin	35 (81.4)	15 (62.5)	28 (87.5)
Gentamicin	16 (37.2)	9 (37.5)	18 (56.2)
Cefotaxime	2 (4.7)	4 (16.7)	NT
Ceftazidime	NT	NT	18 (56.2)
Cefoperazone/sulbactam	28 (65.1)	20 (83.3)	22 (68.8)
Piperacillin/tazobactam	NT	NT	19 (59.4)
Imipenem	35 (81.4)	21 (87.5)	25 (78.1)
Meropenem	26 (60.5)	16 (66.7)	19 (59.4)
Ertapenem	25 (58.1)	9 (37.5)	IR
Ciprofloxacin	NT	NT	5 (15.6)
Cotrimoxazole	10 (23.3)	8 (33.3)	IR

Values are number of susceptible isolates (%). NT = not tested or not reported. IR = intrinsically resistant; not reported. Susceptibility was determined by the Kirby-Bauer disk diffusion method. CLSI does not provide interpretive criteria for cefoperazone/sulbactam (see Material and Methods).

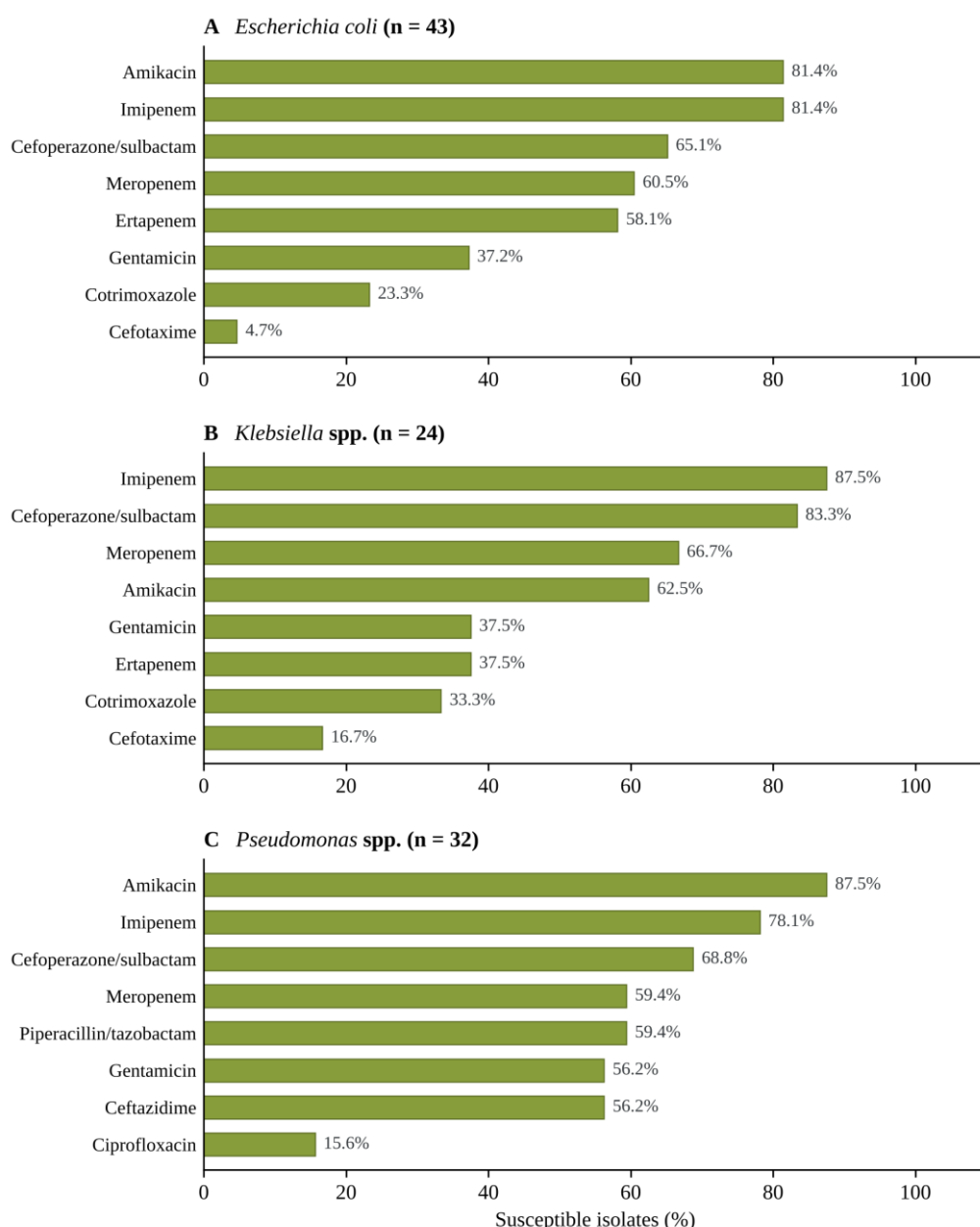


Figure 3. Antimicrobial susceptibility of the predominant Gram-negative isolates from bile. A. *Escherichia coli*. B. *Klebsiella* spp. C. *Pseudomonas* spp. Values beside the bars are the percentage of susceptible isolates.

Klebsiella spp. were most susceptible to imipenem (87.5%), followed by cefoperazone/sulbactam (83.3%), meropenem (66.7%) and amikacin (62.5%). Lower susceptibility was observed for gentamicin and ertapenem (37.5% each), cotrimoxazole (33.3%) and cefotaxime (16.7%).

Susceptibility results were available for 32 of the 33 *Pseudomonas* isolates. Susceptibility was highest to amikacin (87.5%), followed by imipenem (78.1%), cefoperazone/sulbactam (68.8%), meropenem and piperacillin/tazobactam (59.4% each), and gentamicin and ceftazidime (56.2% each). Susceptibility to ciprofloxacin was 15.6%.

When the three organisms were considered together (99 isolates), imipenem was the most active agent (81 isolates; 81.8%; 95% CI 73.1-88.2), followed by amikacin (78; 78.8%; 95% CI 69.7-85.7), cefoperazone/sulbactam (70; 70.7%; 95% CI 61.1-78.8), meropenem (61; 61.6%) and gentamicin (43; 43.4%). Among the 67 isolates of *E. coli* and *Klebsiella* spp., only six (9.0%; 95% CI 4.2-18.2) were susceptible to cefotaxime and 18 (26.9%) to cotrimoxazole, whereas 25 (37.3%; 95% CI 26.7-49.3) were not susceptible to meropenem and 33 (49.3%; 95% CI 37.7-60.9) were not susceptible to ertapenem.

Antimicrobial susceptibility of *Enterococcus* spp.

All 13 *Enterococcus* isolates were susceptible to vancomycin and teicoplanin. Nine isolates (69.2%) were susceptible to high-level gentamicin and five (38.5%) to high-level streptomycin. Six isolates (46.2%) were susceptible to each of amoxicillin/clavulanate and ampicillin, and four (30.8%) to each of penicillin and piperacillin/tazobactam (Figure 4). Because the isolates were not identified to species level, these results cannot be separated for *E. faecalis* and *E. faecium*. Susceptibility to piperacillin/tazobactam was lower than to ampicillin,

which is not expected because ampicillin susceptibility predicts piperacillin/tazobactam susceptibility in enterococci that do not produce beta-lactamase; the results are reported as recorded.

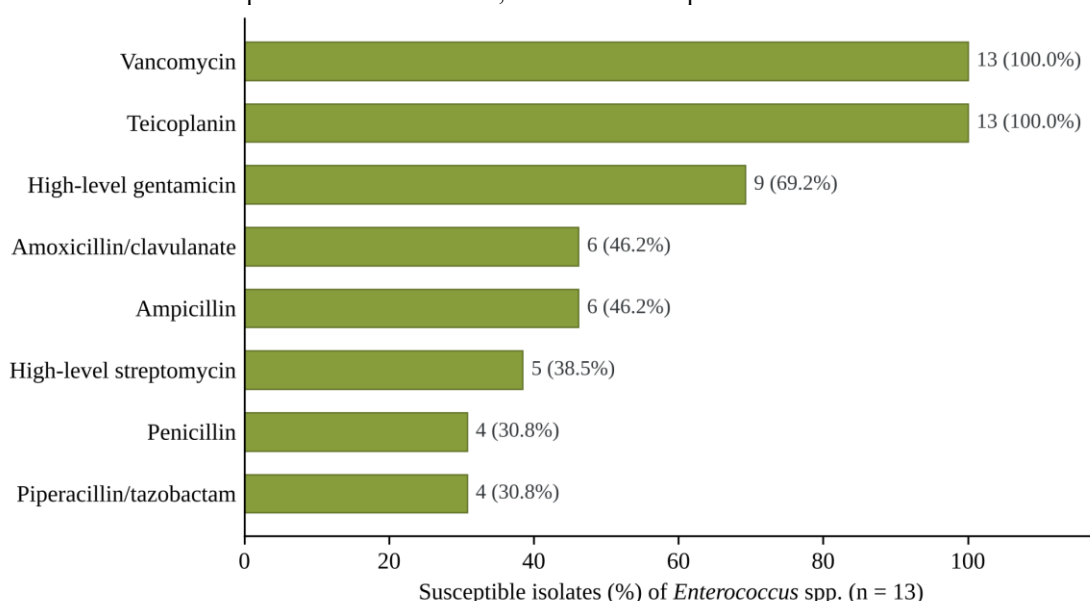


Figure 4. Antimicrobial susceptibility of *Enterococcus* spp. isolated from bile (n = 13). Values beside the bars are number of susceptible isolates (percentage).

Patient characteristics, predisposing factors and complications

Complete demographic and clinical data were available for 75 patients (Table 2). Forty-six (61.3%) were male and 29 (38.7%) were female, giving a male-to-female ratio of 1.6:1. Cholangitis was the most common recorded diagnosis (38; 50.7%), followed by common bile duct obstruction (3; 4.0%) and ulcerative colitis (1; 1.3%). The remaining 33 patients (44.0%) had other hepatobiliary diagnoses or no specific diagnosis recorded.

Table 2. Demographic characteristics, clinical diagnosis, predisposing factors and complications of patients with complete records (n = 75).

Characteristic	Number of patients (%)
Sex	
Male	46 (61.3)
Female	29 (38.7)
Clinical diagnosis	
Cholangitis	38 (50.7)
Common bile duct obstruction	3 (4.0)
Ulcerative colitis	1 (1.3)
Other or not recorded	33 (44.0)
Predisposing factor^a	
Cholelithiasis	34 (45.3)
Carcinoma	23 (30.7)
Biliary stent	22 (29.3)
Liver cirrhosis	2 (2.7)
Complication	
Sepsis	3 (4.0)
Multiple organ dysfunction syndrome	2 (2.7)

^aCategories are not mutually exclusive; some patients had more than one predisposing factor.

Cholelithiasis was the most frequent predisposing factor (34; 45.3%), followed by carcinoma (23; 30.7%), an indwelling biliary stent (22; 29.3%) and liver cirrhosis (2; 2.7%). Several patients had more than one predisposing factor. Complications were uncommon: sepsis developed in three patients (4.0%) and MODS in two (2.7%). Sepsis and MODS were recorded as documented by the treating clinicians; no standard definition could be applied retrospectively.

DISCUSSION

This study describes the organisms recovered from bile over one year in a tertiary care hospital in coastal Karnataka, their susceptibility to the antimicrobial agents in routine use, and the clinical setting in which they occurred. Three findings stand out. Two-thirds of bile samples yielded growth. Gram-negative bacilli, led by *E. coli*, *Pseudomonas* spp. and *Klebsiella* spp., made up almost four-fifths of the isolates. Susceptibility to cefotaxime among the Enterobacterales was very low, and more than one-third of *E. coli* and *Klebsiella* isolates were already non-susceptible to meropenem, whereas amikacin and imipenem retained the most activity.

Bile culture positivity

Bile culture was positive in 67.1% of samples. Reported rates vary widely with the population studied and the route of bile collection: 44% in a series from North India (Gupta et al., 2015), 50.5% among patients undergoing endoscopic retrograde cholangiography in Turkey (Kaya et al., 2012), 54% in an earlier series of patients with cholangitis from Mangalore (Shenoy et al., 2014), 60.2% and 64% in two recent Indian series (Parappanavar et al., 2023; Gupta et al., 2024), 74.8% in patients with acute cholangitis in China (Liu and Zhang, 2014), and 90% or more in patients with cholangitis, choledocholithiasis or therapeutic biliary endoscopy (Sahu et al., 2011; Gargouri et al., 2015; Zhang et al., 2024). Our figure lies in the middle of this range (Table 3 and Figure 5). The highest rates probably reflect selection of patients with established cholangitis or biliary drainage, in whom bacteriobilia is almost universal (Zimmer and Lammert, 2015).

Table 3. Bile culture positivity and predominant isolates in the present study and in previously published series.

Study	Country	Population	Culture positivity	Predominant isolates
Sahu et al. (2011)	India	Acute cholangitis; 95 bile cultures	92.6%	<i>E. coli</i> predominant
Kaya et al. (2012)	Turkey	91 patients undergoing ERC	50.5%	<i>E. coli</i> 28.2%, <i>Pseudomonas</i> 17.3%, <i>Stenotrophomonas maltophilia</i> 15.2%
Kwon et al. (2013)	Korea	1403 patients; 3425 isolates	NR	<i>Enterococcus</i> 22.7%, <i>Escherichia</i> 13.2%, <i>Pseudomonas</i> 10.9%, <i>Klebsiella</i> 10.3%
Liu and Zhang (2014)	China	230 patients with acute cholangitis	74.8%	Gram-positive 57.0%, Gram-negative 40.5%, fungi 2.5%
Shenoy et al. (2014)	India	348 patients with cholangitis	54%	ESBL in 66-73% of <i>E. coli</i> and <i>Klebsiella</i>
Gargouri et al. (2015)	Tunisia	44 patients undergoing biliary drainage	93%	<i>E. coli</i> 26.8%, <i>Enterococcus</i> 17%, <i>Klebsiella</i> 14.6%
Gupta et al. (2015)	India	50 suspected biliary infections	44%	<i>E. coli</i> 40%, <i>Klebsiella</i> 20%, <i>Pseudomonas</i> 16%
Parappanavar et al. (2023)	India	50 patients with biliary obstruction	64%	<i>E. coli</i> , <i>Klebsiella</i> , <i>Pseudomonas</i>
Gupta et al. (2024)	India	226 bile samples	60.2%	Gram-negative predominant; ESBL about 80%
Zhang et al. (2024)	China	880 patients with choledocholithiasis	90.3%	<i>E. coli</i> 30.4%; Gram-negative 56.3%, Gram-positive 41.9%
Cozma et al. (2025)	Romania and France	206 patients with acute cholangitis	NR	Romania: <i>E. coli</i> 30.7%, <i>Pseudomonas</i> 27.8%; France: <i>E. faecalis</i> 15.5%, <i>E. coli</i> 11.3%
Present study	India	161 bile samples	67.1%	<i>E. coli</i> 30.9%, <i>Pseudomonas</i> 23.7%, <i>Klebsiella</i> 17.3%, <i>Enterococcus</i> 9.4%

ERC = endoscopic retrograde cholangiography; ESBL = extended-spectrum beta-lactamase; NR = not reported. Values are taken from the published abstracts of the studies cited. Studies are listed in order of year of publication.

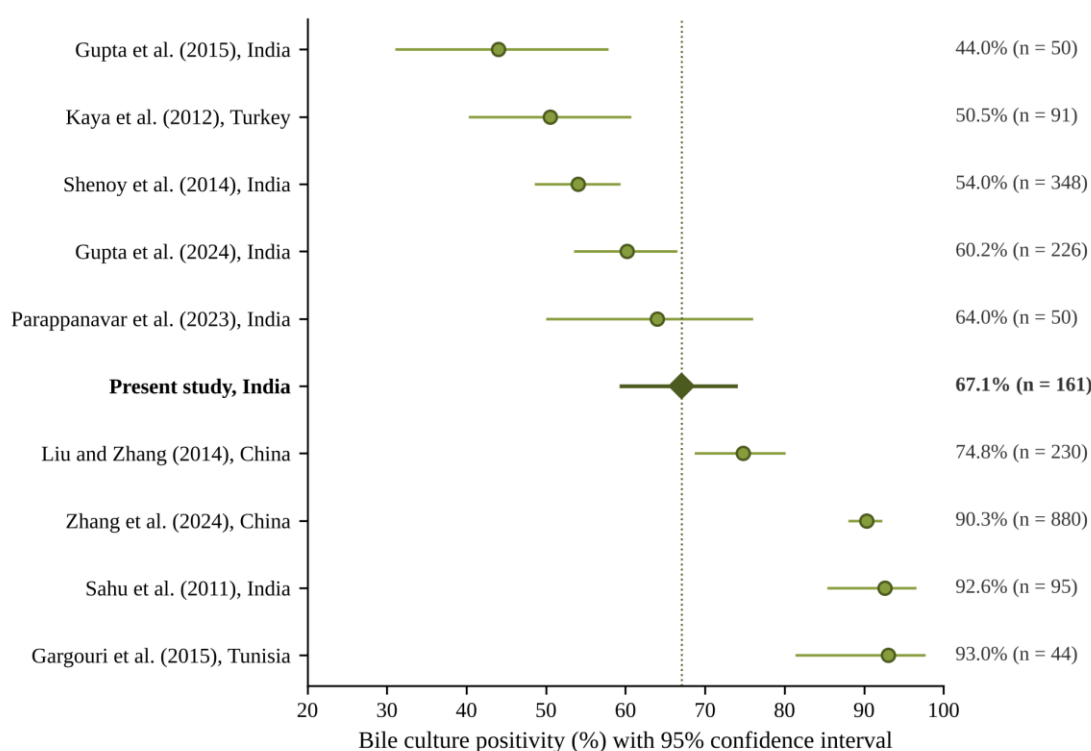


Figure 5. Bile culture positivity in the present study and in previously published series. Points show the reported positivity and horizontal lines the 95% confidence intervals, calculated by the Wilson score method from the reported percentage and number of patients or samples in each study. The dotted line marks the present study.

Although bile is normally sterile, bacteria may enter and multiply within the biliary tract when bile flow is obstructed or when the biliary system is instrumented during diagnostic or therapeutic procedures (Park et al., 2014). The 139 isolates from 108 positive samples in our series indicate that polymicrobial growth was not unusual. Polymicrobial bile cultures were found in 31.5% of culture-positive patients in the earlier Mangalore series (Shenoy et al., 2014) and in 69.5% of positive bile cultures from patients with acute cholangitis in South India (Sahu et al., 2011).

Spectrum of isolates

E. coli was the most frequent isolate (30.9%), followed by *Pseudomonas* spp., *Klebsiella* spp. and *Enterococcus* spp. This agrees with previous studies in which Gram-negative enteric bacilli predominated and *E. coli* was the leading isolate (Bae et al., 2008; Sahu et al., 2011; Salvador et al., 2011). The proportion of *E. coli* is close to the 28.2% reported from Turkey (Kaya et al., 2012) and the 26.8% from Tunisia (Gargouri et al., 2015), and lower than the 40% reported from North India (Gupta et al., 2015). Recent data are similar: *E. coli* made up 30.4% of bile isolates in a large Chinese series (Zhang et al., 2024) and 30.7% in a Romanian centre (Cozma et al., 2025).

Pseudomonas spp. ranked second in our series (23.7%), a higher proportion than the 17.3% in Turkey (Kaya et al., 2012), the 16% in North India (Gupta et al., 2015) and the 10.9% in Korea (Kwon et al., 2013), but close to the 27.8% recently reported from a Romanian centre, where *Pseudomonas* spp. also ranked second after *E. coli* (Cozma et al., 2025). *Pseudomonas* is a recognized healthcare-associated biliary pathogen. Nearly one-third of our patients with clinical records had a biliary stent and a similar proportion had carcinoma, and prior instrumentation in such patients is a plausible explanation for the prominence of this organism, although the retrospective design did not allow isolates to be linked to individual risk factors.

Enterococcus spp. accounted for 9.4% of isolates. Enterococci have been reported with increasing frequency in bile, particularly in complicated, recurrent or healthcare-associated infections (Gargouri et al., 2015; Zimmer and Lammert, 2015), and in some centres they are now the commonest biliary isolate (Kwon et al., 2013; Liu and Zhang, 2014). Gram-positive cocci accounted for 41.9% of isolates in a recent Chinese series and increased over the five years studied (Zhang et al., 2024), and *Enterococcus faecalis* was the leading isolate in a French centre (Cozma et al., 2025). Gram-positive organisms were much less prominent in our setting. Bile culture results should in any case be interpreted with caution, because not every organism isolated from bile represents active infection. This applies particularly to some Gram-positive organisms, which may reflect colonization or contamination rather than invasive infection (Park et al., 2014). The clinical significance of an isolate should therefore be assessed together with the symptoms, laboratory findings, imaging results and evidence of biliary obstruction or inflammation.

Candida spp. were isolated from two samples. Two *Candida* isolates were also reported in the earlier series from Mangalore, whose authors noted that antifungal therapy may be needed when a patient fails to respond to biliary decompression and antibacterial treatment (Shenoy et al., 2014).

Antimicrobial susceptibility of Gram-negative isolates

Amikacin and imipenem showed the highest activity against *E. coli*, and imipenem and cefoperazone/sulbactam against *Klebsiella* spp. Across the three main Gram-negative organisms, about four-fifths of isolates were susceptible to imipenem and to amikacin. These findings are comparable with previous studies. In Turkey, 86% of bile isolates were susceptible to meropenem and to amikacin and 79% to imipenem (Kaya et al., 2012). In Tunisia, all isolates tested were susceptible to imipenem and 89.6% to amikacin (Gargouri et al., 2015). In North India, Gram-negative isolates showed high susceptibility to amikacin and carbapenems and low susceptibility to other agents (Gupta et al., 2015). More recent Indian data also show preserved aminoglycoside activity, with 95.7% of Gram-negative isolates susceptible to aminoglycosides in one series (Gupta et al., 2024) and 75% susceptible to amikacin in another (Parappanavar et al., 2023).

In contrast, susceptibility to cefotaxime was very low: 4.7% in *E. coli* and 16.7% in *Klebsiella* spp. Susceptibility to cotrimoxazole in these organisms and to ciprofloxacin in *Pseudomonas* spp. was also low. Cefotaxime susceptibility of only 14% and ciprofloxacin susceptibility of about 50% were reported in Turkey (Kaya et al., 2012), amoxicillin/clavulanate and ofloxacin were considered unsuitable in Tunisia (Gargouri et al., 2015), and high resistance of *E. coli* to third-generation cephalosporins and ciprofloxacin was found in South India (Sahu et al., 2011). In a recent series from Telangana, only 12.5% of isolates were susceptible to cefixime and 31.3% to ciprofloxacin (Parappanavar et al., 2023). Third-generation cephalosporins, which have traditionally been favoured for biliary infection because of their biliary excretion, can therefore no longer be relied upon as empirical monotherapy in our setting. Ciprofloxacin results were available only for *Pseudomonas* spp., so the activity of fluoroquinolones against our Enterobacterales could not be assessed.

In our data, 39.5% of *E. coli* and 33.3% of *Klebsiella* isolates were not susceptible to meropenem, and 41.9% and 62.5%, respectively, were not susceptible to ertapenem. Carbapenem non-susceptibility of this order among Enterobacterales suggests that carbapenemase producers were present and not only ESBL producers, and it means that carbapenems cannot be assumed to be reliable empirical agents in this setting. Susceptibility to imipenem was higher than to meropenem in both organisms, which is the reverse of the usual relationship between these agents in Enterobacterales. With disk diffusion alone, and without phenotypic or molecular tests for carbapenemases, it is not possible to say whether this discordance is real or reflects a testing or recording artefact. The imipenem results should therefore not be taken as evidence that carbapenems remained fully active, and the carbapenem findings should be confirmed by a reference method in future work.

ESBL-producing organisms

The detection of ESBL-producing *Klebsiella* spp. and *E. coli* in bile is clinically important. ESBLs confer resistance to third-generation cephalosporins, and the organisms that carry them are frequently resistant to fluoroquinolones, cotrimoxazole and aminoglycosides, which leaves carbapenems as the most reliable agents (Paterson and Bonomo, 2005). The very low cefotaxime susceptibility in our Enterobacterales is consistent with widespread ESBL production, and the reduced susceptibility to meropenem and ertapenem suggests that carbapenemase producers were also present. A high ESBL burden has already been documented in this city, where 66% to 73% of biliary *E. coli* and *Klebsiella* isolates were ESBL producers (Shenoy et al., 2014). The burden appears to have grown since our study period: a recent report from North India found ESBL production in about 80% and carbapenemase production in about 75% of Gram-negative bile isolates (Gupta et al., 2024), whereas ESBL rates of 44.1% in *E. coli* and 38.9% in *K. pneumoniae* were reported from China (Zhang et al., 2024). Carbapenems should be reserved for severe or healthcare-associated infection in order to limit further selection of carbapenem-resistant organisms. Amikacin and cefoperazone/sulbactam were active against most isolates in our series and may offer carbapenem-sparing options for less severe infection, subject to the susceptibility result for the individual isolate; the cefoperazone/sulbactam results, however, rest on interpretive criteria that are not defined by CLSI and should be regarded as provisional.

Pseudomonas and *Enterococcus*

Among *Pseudomonas* spp., susceptibility was highest to amikacin and imipenem, and about three-fifths of isolates were susceptible to piperacillin/tazobactam, meropenem, ceftazidime and gentamicin. Good activity of amikacin, carbapenems, gentamicin and piperacillin/tazobactam against *Pseudomonas* isolates from bile has been reported previously (Salvador et al., 2011). Only 15.6% of our isolates were susceptible to ciprofloxacin, which effectively excludes fluoroquinolones as empirical anti-pseudomonal therapy in this setting. Antimicrobial selection for pseudomonal infection should be guided by the susceptibility result for the individual isolate, because resistance patterns vary considerably between institutions and regions.

All *Enterococcus* isolates were susceptible to vancomycin and teicoplanin, which is consistent with a report from North India in which all Gram-positive bile isolates were susceptible to vancomycin, teicoplanin and linezolid (Gupta et al., 2015). Fewer than half of our isolates were susceptible to ampicillin and about one-third were not susceptible to high-level gentamicin, which limits the use of ampicillin-based and synergistic aminoglycoside regimens. Continued surveillance is important because vancomycin-resistant enterococci have increased over time in bile cultures elsewhere (Kwon et al., 2013), and one-quarter of enterococci from bile were vancomycin-resistant in a recent Indian report (Gupta et al., 2024).

Clinical features, predisposing factors and complications

Males outnumbered females in the clinical analysis set, as has been reported in another Indian series of biliary infection (Gupta et al., 2015). This may reflect differences in the underlying biliary disorders, referral patterns or demographic characteristics of the population served.

Cholangitis was the most common diagnosis. Acute cholangitis is a major clinical manifestation of biliary infection and can progress rapidly to severe systemic infection, particularly when biliary drainage is delayed or inadequate (Bae et al., 2008; Zimmer and Lammert, 2015). Cholelithiasis was the most common predisposing factor (45.3%), which is expected because gallstone disease is recognized as the leading cause of acute cholangitis, while benign biliary strictures and malignant obstruction are further important causes of impaired bile flow and infection (Kimura et al., 2013). In patients with acute cholangitis from South India, choledocholithiasis was present in 62.7% and malignancy in 29.2% (Sahu et al., 2011), and malignancy was present in 36.4% of patients in the earlier Mangalore series (Shenoy et al., 2014). The 30.7% with carcinoma in our series is in line with these figures, and cholelithiasis or choledocholithiasis remained the commonest underlying cause (39%) in a recent North Indian series (Gupta et al., 2024).

Carcinoma and biliary stents were each present in about one-third of patients. Malignant biliary obstruction and prior biliary instrumentation have been shown to increase the risk of bacteriobilia, infection with resistant organisms and other infectious complications (Hochwald et al., 1999; Zimmer and Lammert, 2015). Such patients may require closer monitoring and individualized antimicrobial management.

Sepsis and MODS were recorded in only a small proportion of patients. Biliary infection can nevertheless result in severe sepsis and organ dysfunction when diagnosis is delayed, antimicrobial therapy is inadequate or biliary drainage is not achieved promptly (Gupta et al., 2015; Zimmer and Lammert, 2015). Early recognition, appropriate antimicrobial treatment and timely relief of obstruction remain the essential components of management. In a recent Indian series, in-hospital mortality among patients with bacteriobilia was 6.3% (Parappanavar et al., 2023), which underlines the importance of documenting outcomes in future studies from this centre.

Implications for empirical therapy

Current guidelines recommend that empirical therapy for acute cholangitis and cholecystitis be chosen according to severity and to whether the infection is community-acquired or healthcare-associated, that the local antibiogram be taken into account, and that therapy be de-escalated once culture results are available (Gomi et al., 2018). Our findings support three practical points for this institution. First, bile should be sent for culture whenever it is accessible during drainage or surgery, because two-thirds of samples grew organisms and resistance to commonly used empirical agents was frequent. Second, third-generation cephalosporins alone are unlikely to be adequate empirical therapy, and ciprofloxacin is unsuitable where *Pseudomonas* infection is a concern. Third, amikacin and cefoperazone/sulbactam were active against most Gram-negative isolates in this series and could be considered for less severe infection, subject to local confirmation, with carbapenems reserved for severe infection; because carbapenem non-susceptibility was already present in 2015-2016, carbapenem use should also be guided by culture results. These points apply to the period studied. The rise in carbapenemase-producing and vancomycin-resistant organisms described in later Indian data (Gupta et al., 2024) and the marked differences between centres (Cozma et al., 2025) mean that they should be re-examined against current local data before being applied.

Strengths and limitations

The study included all consecutive bile samples received over a full year and reports susceptibility for each major organism separately, which provides a baseline local antibiogram for this period. It also has limitations. It was retrospective and conducted in a single centre, and the isolates date from 2015 to 2016, so the susceptibility pattern may not reflect the current situation. CLSI breakpoints have since changed, notably with the 2023 revision of aminoglycoside breakpoints for Enterobacterales and *Pseudomonas aeruginosa*, so the amikacin and gentamicin results in particular may overstate current activity; because zone diameters were not available, the results could not be reinterpreted against current criteria. Complete clinical records were available for only 75 patients, which restricted the analysis of predisposing factors and complications and precluded comparison between culture-positive and culture-negative patients. The route of bile collection for individual samples, prior antibiotic exposure and patient outcomes were not available for analysis. The laboratory records available for review summarized results as percentages, so the numbers of isolates were derived from them, and nine isolates (6.5%) were not individually specified. Organisms were identified by conventional biochemical tests, as was routine at the time, without confirmation by an automated or mass-spectrometry system. The edition of the CLSI standard used for interpretation was not recorded, no CLSI interpretive criteria exist for cefoperazone/sulbactam, the number of isolates tested for ESBL production was unavailable, carbapenemase production was not tested, enterococci were not identified to species level, the number of distinct patients contributing samples was unknown and repeat samples could not be excluded, and results for netilmicin and tigecycline were excluded because they could not be verified. A specific diagnosis was not recorded for 33 of the 75 patients. Susceptibility was determined phenotypically by disk diffusion; minimum inhibitory concentrations were not measured, only aerobically isolated bacteria were identified, so anaerobes were not characterized, and the resistance determinants, including the genes encoding ESBLs and carbapenemases, were not characterized by molecular methods. The analysis was descriptive, and no association between predisposing factors and culture positivity or resistance was tested. Molecular characterization of resistance genes in biliary isolates from this region is a logical next step.

CONCLUSIONS

Two-thirds of bile samples in this tertiary care hospital yielded growth. *Escherichia coli* was the predominant organism, followed by *Pseudomonas* spp., *Klebsiella* spp. and *Enterococcus* spp. Resistance to cefotaxime was very high among the Enterobacterales, ESBL producers were present, and more than one-third of *E. coli* and *Klebsiella* isolates were already non-susceptible to meropenem. Imipenem and amikacin showed the highest activity against the major Gram-negative isolates, and vancomycin and teicoplanin remained fully active against *Enterococcus* spp. Cholelithiasis, carcinoma and biliary stenting were the main predisposing factors, and cholangitis was the commonest diagnosis. Because these data date from 2015-2016, they provide a baseline rather than a current guide. Routine bile culture with antimicrobial susceptibility testing, interpreted against current breakpoints and supported by continuous local surveillance, is essential for guiding empirical therapy and antimicrobial stewardship in biliary tract infections.

AUTHOR CONTRIBUTIONS

Laxmi Narayana B: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing - original draft. Roshni R. Rao: Methodology, Validation, Supervision, Writing - review and editing. Both authors read and approved the final manuscript and agree to be accountable for all aspects of the work.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author on reasonable request.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

ACKNOWLEDGMENTS

The authors thank the Central Laboratory, Kasturba Medical College Hospital, Mangalore, for access to the laboratory and hospital records used in this study.

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