

# Clinico-epidemiological characteristics of the biliary samples and their antibiotic susceptibility pattern at a teaching hospital in Northern India

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## ABSTRACT

**Introduction:** Bile is deemed a sterile fluid, with the presence of clinical conditions like cholelithiasis, cholecystitis, previous biliary interventions, biliary strictures, and so on, leading to bile stasis, and increases the chances of bacteriobilia. In this study, we recognize the bacterial spectrum of microorganisms isolated from bile samples, diagnostic parameters, and antibiotic sensitivity patterns. **Methods:** A retrospective observational study was carried out by compiling data from the hospital information system of a tertiary care center from 2021 to 2022 to evaluate biliary infections in patients who underwent surgical procedures related to the biliary tract and associated organs. **Results:** A total of 234 patients' bile samples were included in our study. The mean age of patients was  $48.04 \pm 14.74$  years, with more patients below the age of 65 years among those with infected bile samples. One hundred and sixty-three (163/234, 69.66%) patients infected by 209 pathogenic microorganisms were recognized. The most common microorganism isolated was *Escherichia coli* (83/209, 39.71%), followed by *Pseudomonas aeruginosa* (37/209, 17.7%). *Acinetobacter baumannii* and *Klebsiella pneumoniae* isolate owed to more than 90% penicillin, extended-spectrum beta-lactamase, carbapenem, and fluoroquinolone resistance among all isolates. Length of hospital stay, malignant obstruction, and chronic kidney disease were identified as statistically significant risk factors that lead to the isolation of multi-drug-resistant isolates from bile culture. **Conclusion:** We recognized the spectrum of pathogens causing biliary tract infections at our center along with the antibiotic resistance pattern to guide and facilitate prompt and appropriate treatment by primary health care professionals and family medicine practitioners.

**Keywords:** Antibiotic prophylaxis, bile, biliary tract, cholelithiasis, chronic kidney disease

## Introduction

The bile is deemed a sterile fluid, but its stasis could lead to obstruction of the biliary tree, leading to increased chances of cholangitis.<sup>[1]</sup> The presence of clinical conditions like cholelithiasis, cholecystitis, previous biliary interventions, biliary

strictures, primary sclerosing cholangitis, and cholangiopathy and the presence of biliary parasites increase the chances of bacteriobilia.<sup>[2]</sup> The clinical presentation is diverse and can vary from a local biliary infection to an advanced disease, which leads to complications such as sepsis, multiple organ dysfunction syndromes, and death.<sup>[3,4]</sup> The continuous flushing action of bile and the bacteriostatic effects of bile salts maintain the sterility of the biliary tree, and a biliary infection is unexpected under normal conditions. However, any obstruction in the biliary system could be deemed responsible for the colonization and replication of microorganisms, which eventually spreads to the blood and causes systemic infections and sepsis, leading to death.<sup>[4]</sup>

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Biliary infection is common and has been associated with invasive clinical procedures, septicemia, intestinal barrier dysfunction, and gut bacteria translocation.<sup>[5]</sup> Biliary infections remain an important cause of death in patients with severe acute pancreatitis (SAP).<sup>[6]</sup> Therefore, from patients suffering from acute pancreatitis (AP), cholecystitis, and primary biliary cirrhosis, bile samples are frequently sent for clinical microbiological culture tests.<sup>[7]</sup> However, the bile bacterial spectrum and antibiotic resistance characteristics are constantly emerging. Gram-negative bacilli, which are some of the most common opportunistic pathogens in hospitals, have shown increasing trends toward drug resistance in recent years.<sup>[8]</sup> Gram-negative bacilli mainly include isolates from the Enterobacteriaceae family and other non-lactose fermenting bacteria. Bacterial isolates often produce efflux pumps, induce position changes and enzyme production, develop various drug resistance mechanisms, and easily evolve into multi-drug-resistant (MDR) organisms (MDROs) that are resistant to three or more types of clinical antibiotics used at the same time.<sup>[9]</sup> In recent years, with the extensive use of antibiotics for the treatment of bacterial infections, MDROs have become common and important pathogens responsible for nosocomial infections, resulting in an intractable challenge for clinical diagnosis and treatment<sup>[10]</sup>; therefore, it is necessary to identify and combat the risk factors for MDRO's infection in patients.

This study highlights an important need among clinicians and family physicians to identify the bacterial spectrum of the microorganisms isolated from the bile samples, diagnostic parameters, and antibiotic susceptibility patterns in patients. We also investigated the risk factors related to the acquisition of MDR biliary infections and promote the rational use of antibiotics to control MDR bacterial infections.

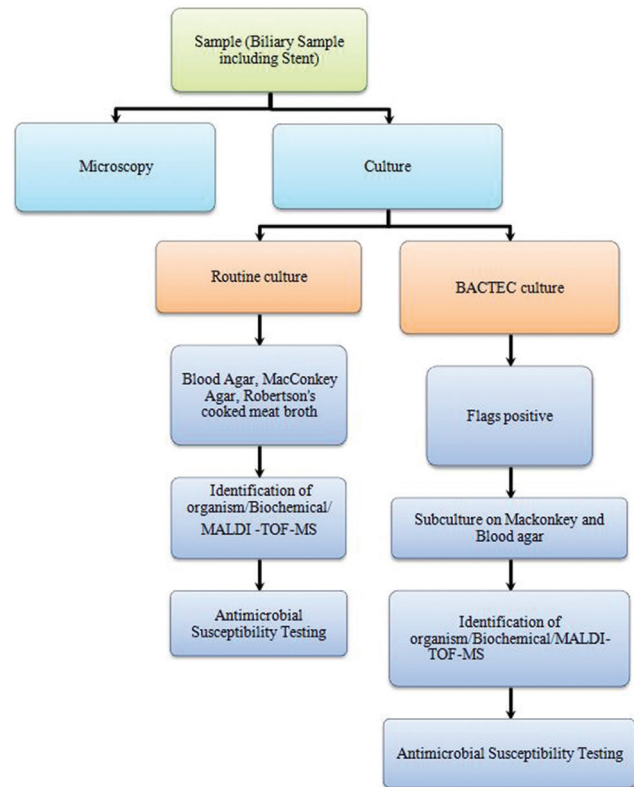
## Material and Methods

This study was carried out retrospectively in the Bacteriology section of the Department of Microbiology at a teaching hospital in northern India from July 2021 to July 2022 for one year. The study was approved by the institute ethics committee (2021-52-EMP-EXP dated 13/04/2021).

**Inclusion criteria:** Our patient group included all adults admitted to the post-operative ward of the Department of Surgical Gastroenterology who underwent surgical procedures related to the biliary tract and associated organ systems.

**Exclusion criteria:** All samples that were not labeled properly, contaminated, and delayed in sample transport for more than 2 hours were excluded from our study.

A total of 234 bile samples were included in our study and processed according to the laboratory protocol. Figure 1 explains the flow of sample processing performed at our laboratory. Initially, a Gram-stained smear was prepared for all the samples, and blood agar and Mac Conkey agar plates were inoculated and further incubated at 37°C for 48 h. Microscopic features,



**Figure 1:** Flowchart of the workflow followed in our laboratory

characteristics of the colony obtained on culture plates, and biochemical tests were used to identify and characterize the bacterial isolates.<sup>[11]</sup>

## Antibiotic susceptibility testing

Antibiotic discs containing amikacin (30 mcg), ampicillin (10 mcg), ampicillin–sulbactam (10/10 mcg), aztreonam (30 mcg), ceftazidime (30 mcg), ceftriaxone (30 mcg), cefoperazone–sulbactam (75/10 mcg), ciprofloxacin (5 mcg), cefoxitin (30 mcg), clindamycin (2 mcg), doxycycline (10 mcg), ertapenem (10 mcg), gentamicin (10 mcg), erythromycin (15 mcg), imipenem (10 mcg), levofloxacin (5 mcg), linezolid (30 mcg), meropenem (30 mcg), minocycline (30 mcg), tigecycline (15 mcg), trimethoprim–sulfamethoxazole (1.25/23.75 mcg), teicoplanin (30 mcg), piperacillin–tazobactam (100/10 mcg), vancomycin (30 mcg), and colistin (0.016–256 mcg) were used. Epsilometric test strips were procured from the company bioMérieux and were used according to the instructions of the manufacturer. Kirby Bauer's disc diffusion method was used to perform the antibiotic sensitivity testing using an epsilometric test strip and disc diffusion test, in compliance with the norms of the Clinical and Laboratory Standards Institute.<sup>[12]</sup> While performing the antibiotic susceptibility testing, inoculums with a turbidity of 0.5 McFarland were prepared for the bacterial isolate and it was lawn cultured on Muller–Hinton agar plates, following which the antibiotic discs and E-strips were placed on the inoculated plates and incubated at 37° C overnight. CLSI guidelines were used to classify the antibiotics as sensitive, intermediate, and resistant.<sup>[12]</sup>

A record of all the diagnostic parameters was extracted from the hospital information system. Statistical analyses were performed using the software program IBM SPSS Statistics version 20.0 (SPSS Inc.), with  $P < 0.05$ , which was considered statistically significant.

### Result

A total of 234 patients who underwent a surgical procedure in the Department of Surgical Gastroenterology, followed by admission to their ward, were included in our study. The most common cause of undergoing surgical procedures was cholelithiasis (129/234, 55.13%), followed by malignancy (118/234, 50.43%) and complicated pancreatitis (10/234, 4.27%). We observed a predominance of female patients with 61.11% (143/234, 61.11%) female patients and 38.89% (91/234, 38.89%) male patients. The mean age of the patients was  $48.04 \pm 14.74$  years, with a significant preponderance of patients below the age of 65 years among those with infected bile samples in comparison to those with sterile bile samples [Table 1].

The most common cause of obstruction to the flow of bile through the biliary tree was identified as the presence of indwelling biliary drainage in 74.36% (174/234, 74.36%) patients after

surgery, followed by cholecystitis in 61.11% (143/234, 61.11%) patients, procedures involving biliary-enteric anastomosis in 58.12% (136/234, 58.12%), which mainly includes the roux-en-y biliary-enteric anastomosis technique, and cholelithiasis in 51.28% (120/234, 51.28%) patients. The occurrence of biliovascular injuries, benign strictures, indwelling biliary drainage, and cholelithiasis among the patients with infected bile samples was a statistically significant risk factor for infection in comparison to patients with sterile bile samples [Table 1].

The most common co-morbidity identified among our study cohort included was recognized as diabetes mellitus in 99 (99/234, 42.31%) patients, followed by hypoproteinemia in 97 (97/234, 41.45%) patients, chronic liver disease in 76 (76/234, 32.48%) patients, and hypothyroidism in 74 (74/234, 31.62%) patients. Hypothyroidism was identified as a significant risk factor in patients with infected bile samples in comparison to those with sterile bile samples. Table 1 also describes the significant risk of death in patients with biliary infections.

We also compared the diagnostic parameters among the patients with infected bile samples and those with sterile bile samples, as described in Table 2. Procalcitonin was found to marginally rise in patients with infection in comparison to those without infection.

**Table 1: Comparison of demographic factors among those with infected and sterile bile samples (n=234)**

Observation parameters	Total bile samples (n=234)	Infected bile samples (n=163)	Sterile bile samples (n=71)	P	Confidence interval (95% CI)
Gender					
Male	91 (38.89%)	60 (36.81%)	31 (43.66%)	0.323	1.24–1.44
Female	143 (61.11%)	103 (63.19%)	40 (56.34%)	0.323	1.20–1.35
Age					
<65 years	201 (85.89%)	145 (88.96%)	56 (78.87%)	0.042*	1.21–1.34
≥65 years	33 (14.10%)	18 (11.04%)	15 (21.13%)	0.042*	1.28–1.63
Causes of obstruction					
Biliovascular injury	45 (19.23%)	38 (23.31%)	7 (9.86%)	0.016*	1.05–1.27
Malignant obstruction	119 (50.85%)	80 (49.08%)	39 (54.93%)	0.411	1.24–1.41
Benign stricture	56 (23.93%)	33 (20.25%)	23 (32.39%)	0.045*	1.28–1.54
Indwelling biliary drainage	174 (74.36%)	128 (78.53%)	46 (64.79%)	0.027*	1.19–1.33
Cholecystitis	143 (61.11%)	104 (63.80%)	39 (54.93%)	0.2	1.19–1.34
Choledochal cyst	31 (13.25%)	24 (14.72%)	7 (9.86%)	0.313	1.05–1.35
Biliary-enteric anastomosis	136 (58.12%)	91 (55.83%)	45 (63.38%)	0.230	1.25–1.41
Cholelithiasis	120 (51.28%)	92 (56.44%)	28 (39.44%)	0.017*	1.15–1.30
Underlying co-morbidities					
Diabetes mellitus	99 (42.31%)	72 (44.17%)	27 (38.03%)	0.382	1.18–1.36
Hypothyroidism	74 (31.62%)	62 (38.04%)	12 (16.90%)	0.001*	1.08–1.25
Hypoproteinemia	97 (41.45%)	65 (39.88%)	32 (45.07%)	0.459	1.23–1.43
Chronic liver disease	76 (32.48%)	51 (31.29%)	25 (35.21%)	0.556	1.22–1.44
Immunosuppression	19 (8.12%)	13 (7.98%)	6 (8.45%)	0.903	1.09–1.55
Chronic kidney disease	44 (18.80%)	34 (20.86%)	10 (14.08%)	0.223	1.10–1.36
Length of hospital stay (in days)	19.33±11.89	19.63±11.86	18.65±12.04	0.56	0.887–1.268
ICU stay					
Yes	33 (14.10%)	24 (14.72%)	9 (12.68%)	0.679	17.79–21.46
No	201 (85.89%)	139 (85.28%)	62 (87.32%)	0.679	15.80–21.50
Outcome					
Alive	209 (89.32%)	141 (86.50%)	68 (95.77%)	0.035*	1.26–1.39
Dead	25 (10.68%)	22 (13.49%)	3 (4.22%)	0.035*	0.98–1.26

\* $P \leq 0.05$  is statistically significant

Abdominal cancers were identified among 118 (118/234, 50.43%) patients in our study. Conjugated bilirubin levels, total bilirubin levels, and total leukocytes were found to be significantly raised among patients with infected bile samples in comparison to those with sterile bile samples.

One hundred and sixty-three (163/234, 69.66%) patients of biliary infection were recognized in patients included in our study group. Out of the 163 patients suffering from biliary infections, 44 (44/163, 26.99%) patients were found to be suffering from polymicrobial infections and 119 (119/163, 73.01%) patients were found to be suffering from monomicrobial infections [Figure 2]. Two hundred and nine bacterial microorganisms were identified to infect the 163 patients. The most common microorganism isolated from the infected bile samples was *Escherichia coli* (83/209, 39.71%), followed by *Pseudomonas aeruginosa* (37/209, 17.7%) and *Klebsiella pneumoniae* (28/209, 13.39%). *Enterococcus species* (35, 16.75%) was identified as the most common Gram-positive coccus isolated from bile samples included in our cohort [Figure 3].

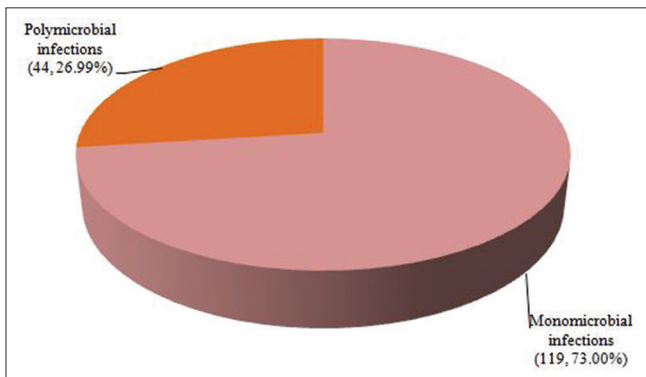
After antibiotic sensitivity testing, 105 (105/209, 50.24%) bacterial isolates were found to be MDR. The incidence of aminoglycoside resistance among the 170 (170/209, 81.34%) Gram-negative isolates was 42.94% (73/170, 42.94%). Aminoglycoside resistance was recognized most commonly among *Klebsiella pneumoniae* isolates (23/73, 31.51%) and *Escherichia coli* (23/73, 31.51%), followed by *Pseudomonas aeruginosa* (13/73, 17.80%) and *Morganella morganii* (5/73, 6.85%). Three *Acinetobacter baumannii*

isolates were obtained from the infected bile cultures, and 100% (3/3, 100%) resistance to aminoglycosides was recognized among them.

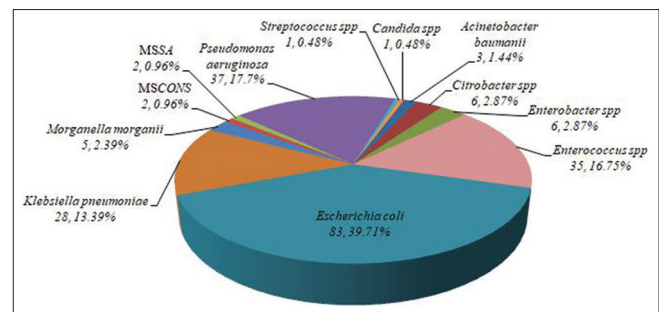
Beta-lactamase resistance was predominantly recognized among the Gram-negative isolates. An incidence of 80.86% (169/209, 80.86%) beta-lactamase resistance was reported among the microorganisms. *Escherichia coli* (74/169, 43.79%) was identified as the most beta-lactam-resistant isolate, followed by *Klebsiella pneumoniae* (28/169, 16.57%) and *Pseudomonas aeruginosa* (19/169, 11.24%). Among the above-mentioned isolates, *Klebsiella pneumoniae* was found 100% (28/28, 100%) resistant, *Escherichia coli* was found 89.16% (74/83, 89.16%) resistant, and *Pseudomonas aeruginosa* was found 50% (19/37, 51.35%) resistant to beta-lactam antibiotics.

Extended-spectrum beta-lactamase (ESBL) resistance was another well-recognized property among the Gram-negative isolates in our study cohort. One hundred and forty-two (142/209, 67.94%) isolates were found resistant to ESBL antibiotics. *Escherichia coli* (58/142, 40.84%) was identified as the most ESBL-resistant isolate, followed by *Klebsiella pneumoniae* (27/142, 19.01%) and *Pseudomonas aeruginosa* (18/142, 12.68%). Among the above-mentioned isolates, *Klebsiella pneumoniae* was found 96.43% (27/28, 96.43%) resistant, *Escherichia coli* was found 69.88% (58/83, 69.88%) resistant, and *Pseudomonas aeruginosa* was found 47.37% (18/37, 48.65%) resistant to ESBL antibiotics.

Carbapenem resistance has been identified as an emerging problem among bacterial pathogens from various sites of infection. Ninety-six (96/209, 45.93%) bacterial pathogens



**Figure 2:** Distribution of cases of infected bile cultures based on the number of microbes isolated (n = 163)



**Figure 3:** Microorganisms isolated from the infected bile samples included in this study (n = 209) MSCONS – Methicillin-sensitive coagulase-negative *Staphylococcus* MSSA – Methicillin-sensitive *Staphylococcus aureus*

**Table 2: Comparison of diagnostic parameters among those with infected and sterile bile samples (n=234)**

Diagnostic parameters	Total bile samples (n=234)	Infected bile samples (n=163)	Sterile bile samples (n=71)	P*	Confidence interval (95% CI)
Procalcitonin (ng/ml) (mean±SD)	2.30±7.97	2.62±8.98	1.58±4.91	0.362	0.659–6.367
CA 19.9 (µg/ml) (Mean±SD)	377.06±1879.68	397.67±2043.06	330.04±1451.37	0.804	0.349–9.021
ALT (SGPT) (mean±SD)	65.85±56.44	67.30±58.39	62.54±51.96	0.550	0.856–1.381
AST (SGOT) (mean±SD)	71.37±50.85	72.75±53.45	68.20±44.55	0.530	0.885–1.299
Conjugated Bilirubin (mg/dl) (mean±SD)	2.63±3.93	2.23±3.16	3.72±5.16	0.0073*	0.412–0.928
Total bilirubin (mg/dl) (mean±SD)	4.24±5.68	3.62±4.65	5.66±7.37	0.0112*	0.452–0.957
Total leukocyte count (cells/cubic mm) (mean±SD)	14496.13±7986.32	15333.82±8963.86	12584.79±4610.11	0.0152*	1.075–1.380

\*P≤0.05 is statistically significant

isolated in this study were found to be resistant to carbapenem antibiotics. *Escherichia coli* (41/96, 42.71%) was identified as the most ESBL-resistant isolate, followed by *Klebsiella pneumoniae* (25/96, 26.04%) and *Pseudomonas aeruginosa* (15/96, 15.63%). Among the above-mentioned isolates, *Klebsiella pneumoniae* was found 89.28% (25/28, 89.28%) resistant, *Escherichia coli* was found 49.39% (41/83, 49.39%) resistant, and *Pseudomonas aeruginosa* was found 39.47% (15/37, 40.54%) resistant to ESBL antibiotics.

Fluoroquinolone resistance was observed among 163 (163/209, 77.99%) pathogenic bacteria identified from the infected bile samples. *Escherichia coli* (70/163, 42.94%) was found most resistant to fluoroquinolones, followed by *Klebsiella pneumoniae* (27/163, 16.56%) and *Pseudomonas aeruginosa* (19/163, 11.66%). Among the above-mentioned isolates, *Klebsiella pneumoniae* was found 96.43% (27/28, 96.43%) resistant, *Escherichia coli* was found 84.34% (70/83, 84.34%) resistant, and *Pseudomonas aeruginosa* was found 50% (19/37, 51.35%) resistant to fluoroquinolone antibiotics.

*Acinetobacter baumannii* was isolated in only three (3/209, 1.44%) cases from the infected bile samples, with resistance to all the first-line antibiotics used against them. Among the second-line drugs, the microorganism was established susceptible to colistin in 66.67% (2/3, 66.67%) of isolates and was recognized susceptible to 33.33% (1/3, 33.33%) of *Acinetobacter baumannii* isolates. Only one (1/3, 33.33%) isolate of *Acinetobacter baumannii* was resistant

to all second-line drugs along with Ceftazidime – avibactam and aztreonam synergy which was absent.

Among Gram-positive cocci, *Enterococcus spp* (35/209, 16.75%) was identified as the most common Gram-positive pathogen recognized among those isolated from infected bile samples. Out of the 35 *Enterococcus spp* isolates, 27 (27/35, 77.14%) isolates were ampicillin-resistant. Four (4/209, 1.91%) causative microorganisms belonging to *Staphylococcus spp* were identified, but none was resistant to ceftoxitin.

We also demonstrated the demographics and risk factors associated with the isolation of MDR bacteria from bile culture in Table 3. MDR pathogenic microorganisms were significantly isolated from individuals with a mean age of 52.50 ± 12.04 years. The most common risk factor responsible for increased isolation of MDR microorganisms from infected bile samples was indwelling biliary drainage [64 (60.95%)], followed by cholecystitis [51 (48.57%)], although none of them were statistically significant risk factors. Malignant obstruction in the biliary system, chronic kidney disease, and increased length of hospital stay were deemed as significant risk factors for the isolation of MDR microorganisms [Table 3].

## Discussion

We identified 234 patients with suspected bacterial infections in our study cohort. The mean age of patients was 48.04 ± 14.74 years

**Table 3: Demographic and risk factors associated with isolation of MDR bacteria from bile culture (n=209)**

Observation parameters	MDR isolates (n=105/209, 50.24%)	Susceptible isolates (n=104/209, 49.76%)	P*	Confidence interval (95% CI)
Age (Mean±SD)	52.50±12.04	44.04±14.55	<0.001*	1.104–1.29
Gender				
Male	31 (29.52%)	29 (27.88%)	0.699	1.35–1.61
Female	50 (47.62%)	51 (49.04%)	0.699	1.42–1.61
Causes of obstruction				
Biliovascular injury	17 (16.19%)	21 (20.19%)	0.485	1.39–1.72
Malignant obstruction	48 (45.71%)	30 (28.85%)	0.004*	1.27–1.50
Benign stricture	12 (11.43%)	21 (20.19%)	0.086	1.46–1.81
Indwelling biliary drainage	64 (60.95%)	63 (60.58%)	0.730	1.41–1.58
Cholecystitis	51 (48.57%)	53 (50.96%)	0.823	1.41–1.61
Choledochal cyst	8 (7.62%)	16 (15.38%)	0.082	1.46–1.87
Biliary-enteric anastomosis	47 (44.76%)	43 (41.35%)	0.469	1.37–1.58
Cholelithiasis	42 (40.0%)	50 (48.08%)	0.237	1.44–1.65
Underlying comorbidities				
Diabetes mellitus	36 (34.28%)	35 (33.65%)	0.819	1.37–1.61
Hypothyroidism	31 (29.52%)	31 (29.81%)	0.950	1.37–1.63
Hypoproteinemia	37 (35.24%)	27 (25.96%)	0.094	1.30–1.55
Chronic liver disease	29 (27.62%)	22 (21.15%)	0.215	1.29–1.57
Immunosuppression	6 (5.71%)	7 (6.73%)	0.790	1.22–1.85
Chronic kidney disease	21 (20.0%)	11 (10.58%)	0.044*	1.17–1.52
Length of hospital stay (in days)	21.73±13.65	17.40±9.48	0.008*	1.061–1.465
Admission to ICU present	13 (12.38%)	11 (10.58%)	0.634	1.24–1.67
Outcome				
Alive	68 (64.76%)	71 (68.27%)	0.624	1.43–1.59
Dead	12 (11.43%)	10 (9.62%)	0.624	1.23–1.68

\*P≤0.05 is statistically significant

with a female predominance (143/234, 61.11%), which agrees with a study conducted by Kaya et al.,<sup>[13]</sup> where the mean age of the patients was  $53.7 \pm 17.5$  years with the female predominance of 73% (66/91, 72.53%). As known in the literature, a healthy individual has a sterile biliary system, but bacterial colonization of the biliary system can be associated with biliary pathology.<sup>[14]</sup> Biliary obstruction is a well-known cause of bacteriobilia.<sup>[13]</sup> Our study cohort identified the most significant cause of obstruction in biliary flow leading to bacteriobilia as the presence of an indwelling biliary drain in 74.36% (174/234, 74.36%) cases, followed by cholelithiasis in 51.28% (120/234, 51.28%) cases, which are in contrast to the studies that suggest that malignant biliary strictures increase risk of colonization but agree with the increase in bacterial infection concerning the presence of indwelling stents and drains.<sup>[15]</sup>

We report positivity in bile cultures of 69.66% (163/234, 69.66%) in this study, which is in agreement with a study conducted by Zhao et al.,<sup>[16]</sup> where the positivity rate of bile culture was 70%, whereas a recent study by Zhao et al.<sup>[17]</sup> reports an even higher positivity of 96.4% due to strict inclusion and exclusion criteria of their study. From the evidence in the literature, the most common intestinal bacteria isolated from infected biliary tracts were *Escherichia coli* among Gram-negative bacteria (GNB) and *Enterococcus spp* among Gram-positive cocci (GPC).<sup>[16,18,19]</sup> In agreement with the studies quoted above, the most common bacterial pathogens isolated from our study were *Escherichia coli* (83/209, 39.71%) among GNB and *Enterococcus spp* among GPC (35/209, 16.75%). We encountered 80.38% (168/209, 80.38%) of GNB, 19.62% (41/209, 19.62%) GPC, and only one isolate of fungi (1/209, 0.5%) in this study, which is per a study by Zhao et al.,<sup>[17]</sup> where positive bile cultures showed predominant growth of GNB (76.8%), followed by GPC (22.5%) and a minority of fungi (0.7%).

A predominance of GPC was identified in studies by Reiter et al.<sup>[20]</sup> and Rupp et al.,<sup>[21]</sup> which can be attributed to the belligerent antibiotic coverage provided for GNB. A rising trend of increased isolation of *Enterococcus spp* from bile cultures was also observed in our study, which was similarly observed in studies from other institutes where the empirical therapy for bacteriobilia commonly covered GNB, leading to the slight increase of Gram-positive *Enterococci spp*.<sup>[16]</sup> The emergence of a newer spectrum of microorganisms over time demands future reviews to characterize the pathogen profile for fruitful antibiotic coverage.<sup>[22,23]</sup>

We recorded a high level of antibiotic resistance among the commonly isolated microorganisms. *Escherichia coli* was most susceptible to amikacin and imipenem among the first-line drugs, while MDR *Escherichia coli* tested for susceptibility to colistin was found 100% (41/41, 100%) susceptible. Higher susceptibility of *Escherichia coli* to amikacin was recorded in a study by Zhou et al.<sup>[16]</sup> The rate of resistance to third-generation cephalosporins among *Escherichia coli* isolates from infected bile samples was recorded in the range of 83.13% to 89.16%, which is very high in comparison to a study by Yun et al.,<sup>[24]</sup> which record a resistance of 20% to 60%.

With soaring resistance to penicillins and cephalosporins, the use of these antibiotics has been practically reduced in severe infections. The extended-spectrum  $\beta$ -lactamase (ESBL) character of a microorganism was found to resist the effect of penicillins and cephalosporins.<sup>[17]</sup> The highest ESBL producing character among microorganisms isolated from the infected bile samples was *Morganella morganii* (5/5, 100%), followed by *Klebsiella pneumoniae* (27/28, 96.43%) and *Escherichia coli* (58/83, 69.88%), which is in agreement with the study by Zhou et al.<sup>[17]</sup> Carbapenems were found to be resistant in approximately 50% of isolates, which is much higher in contrast to a study by Zhou et al.,<sup>[17]</sup> which could be attributed to the rampant use of carbapenems, leading to the slow but steady emergence of carbapenem-resistant microorganisms.<sup>[25]</sup> Clinicians at our institute still prefer to start carbapenems and aminoglycosides in selected cases as empirical treatment for infections as serious as bacteriobilia, despite *in vitro* resistance to the antibiotic after dose adjustment.

As we already know, there is a trend of increasing incidence of *Enterococcus spp* in various studies, which also mirrors the finding of our study.<sup>[20,21]</sup> In agreement with previously published articles, the *Enterococcus spp* isolates were reported to be more than 90% susceptible to linezolid, vancomycin, and teicoplanin.<sup>[16]</sup> Vancomycin was regarded as the drug of choice for the empirical treatment of *Enterococcus spp*, which was also recommended by the Tokyo Guidelines 2018.<sup>[26]</sup>

The use of newer technologies like endoscopic retrograde cholangiopancreatography (ERCP) and other ultrasonographic techniques has helped in testing the bile samples directly from the suspected site of infection. This also helps in the recognition of the antibiotic-resistant pattern of the microbes isolated from the infected bile samples.<sup>[20]</sup> Thus, the antibiotic sensitivity pattern will direct the identification of the antibiotic coverage that needs to be provided for prophylactic treatment of risk factors like surgical procedures of the biliary tract that may lead to biliary tract infection.<sup>[27-30]</sup>

This study facilitates to enhance the knowledge of the general or family physicians for the causative pathogens of bacteriobilia along with their antibiotic susceptibility pattern and also demonstrates the causes of biliary obstruction, co-morbid conditions, and risk factors along with the outcome of patients suffering from MDR bacteriobilia.

Our research was limited by various factors; first, this is a retrospective observational study that identifies the annual incidence of biliary infections at a single tertiary care center. Second, the findings of our study only represent the spectrum of infections and antibiotic resistance at a single center and do not represent the rate of infection in other hospitals of the region. Third, data were only recorded in the hospital's electronic system and the case files from the surgical gastroenterology ward were used for the extraction of clinical data.

## Conclusion

Delayed treatment of bacteriobilia could lead to the development of complications like sepsis, multi-organ failure, or death. Thus, early and appropriate treatment of patients with biliary tract infections is needed. Our study guides physicians in better selection of antibiotics and antibiotic prophylaxis for patients at risk of developing biliary tract infections with MDR pathogens.

## Author contribution

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Data collection: Akanksha Dubey, Mitra Kar, Ankita Tiwari. Data analysis: Chinmoy Sahu, Sangram Singh Patel. Supervision: Chinmoy Sahu, Sangram Singh Patel. Writing - original draft: Mitra Kar. Writing – review & editing: Mitra Kar, Akanksha.

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All authors read and approved the final version of the manuscript.

## Ethical statement

The study protocol was approved by the institutional Ethics Committee of Sanjay Gandhi Postgraduate Institute of Medical Sciences (Reference number 2021-52-EMP-EXP dated 13/04/2021).

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## Conflicts of interest

There are no conflicts of interest.

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