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Original Article

Bacteriological and Antimicrobial Susceptibility Profile in Biliary Tract Infections: A Retrospective Study

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Abstract

Introduction In the past few years, although there has been no change in the bacteriological profile of organisms responsible for biliary tract infections (BTIs), changes in the drug susceptibility pattern of these organisms have been reported.

Material and Methods We planned a retrospective study to evaluate the microbiological profile and antibiotic susceptibility patterns of isolates from patients with BTI. The study was conducted in the Department of Microbiology over a period of 1 year. We recorded the culture positivity rates, organisms identified, and the pattern of antibiotic susceptibility.

Results Out of 226 bile samples received, 60.2% samples were found to be culture positive. Most of the patients were older than 40 years. Hypertension (33.1%), diabetes (28.7%), and cardiovascular diseases (8.1%) were the common underlying diseases. Common patient symptoms were abdominal pain (77.89%), jaundice (42.24%), and fever (41.52%). Cholangitis was present in 20.6% patients. Cholelithiasis or choledocholithiasis was the most common underlying etiology (39%). A total of 142 isolates were obtained, with most patients with a predominance of gram-negative isolates. Tigecycline and aminoglycosides were found to be effective against gram-negative isolates with a susceptibility of 93.4 and 95.7%, respectively. Of the gram-negative isolates, approximately 70% were multidrug resistant (MDR), with approximately 80 and 75% being extended spectrum beta-lactamases (ESBLs) and carbapenemase producers, respectively. Enterococci were 100% susceptible to linezolid and 25% of these were vancomycin-resistant enterococci (VRE).

Conclusion In summary, the predominant bacteria in our study were gram-negative bacteria that showed increased resistance to cephalosporins and carbapenems. This makes the treatment of bile duct infections increasingly difficult. A comprehensive analysis of the bacterial distribution and drug resistance profiles in patients with BTIs helps physicians to make better antibiotic policies for empiric treatment.

Keywords
- gram-negative isolates
- antibiotic susceptibility
- bacterial profile
- biliary tract infections
- clinical profile

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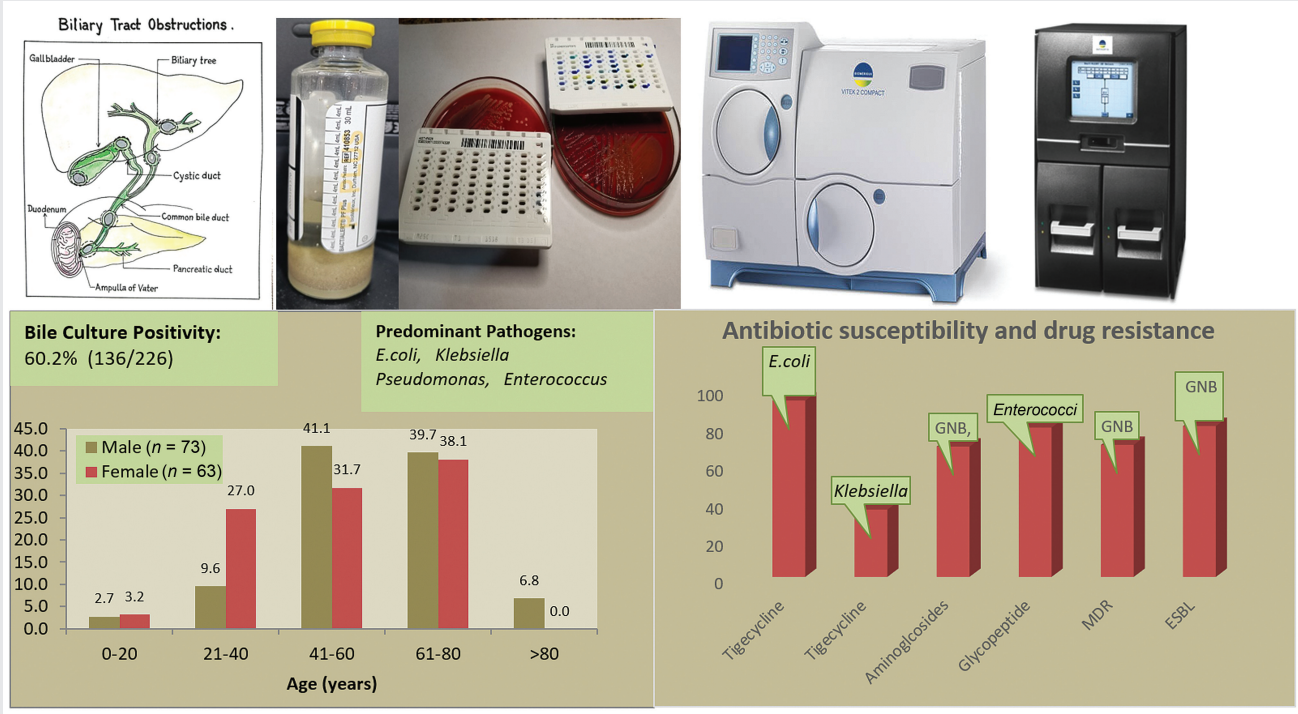
Graphical Abstract



Introduction

Bile is a sterile secretion produced by the liver and stored in the gallbladder. It helps remove microorganisms entering the biliary tract by its flushing action. Bactibilia occurs either due to the presence of stones in the gallbladder or biliary tract.[1,2] Microorganisms from the duodenum contents may enter the gallbladder due to biliary tract obstruction leading to septicemia through the portal venous channel.[3] Eventually, the bacteria presumably translocate into the circulation causing a systemic infection. Acute cholangitis spans a continuous clinical spectrum and can progress from a local biliary infection to advanced disease with sepsis and multiple organ dysfunction syndrome.[2] According to recent studies, biliary tract infections (BTIs) are associated with mortality rates of 9 to 12%.[4] BTIs predominantly have bacterial etiology with gram-negative aerobic flora being the most prevalent. The most commonly isolated aerobic pathogens are *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., *Enterococcus* spp., and *Salmonella* spp.[2] Candida may be isolated in very few of the cases. Additionally, 4 to 5% of BTIs are caused by more than one organism (polymicrobial).[5,6]

In the past few years, although there has been no change in the bacteriological profile of organisms responsible for BTIs, there has been a significant change in the drug susceptibility pattern of these organisms. These infections with increased frequency of extended-spectrum β-lactamase (ESBL) and carbapenemase-producing Enterobacteriaceae (CPE) organisms lead to redundancy of currently used empiric antibiotic therapy.[7,8] Therefore, choosing an antibiotic regimen remains challenging for clinicians.

Therefore, this study aimed to know the microbiological profile and current antibiotic susceptibility patterns of isolates from patients with BTIs admitted to a teaching hospital in North India.

Material and Methods

This retrospective cross-sectional study was conducted in the department of microbiology in a tertiary care hospital. This study was carried out over 1 year from March 2022 to Feb 2023.

Sample Collection

Bile samples were collected by intraprocedural extraction through endoscopic retrograde cholangiopancreatography (ERCP) or during surgery procedures. In case of a complicated or perforated gallbladder, bile was collected from the perihepatic space under aseptic conditions. Depending on the clinical presentation, empirical antibiotics were started before sample collection on the basis of the hospital antibiotic policy. Bile samples were received in the microbiology laboratory for culture and antibiotic sensitivity testing.

Sample Processing

All the bile samples received in the microbiology laboratory were inoculated in blood culture bottles for enrichment and were processed in an automated BACTEC or BacT/ALERT system. Once a blood culture bottle flagged positive, a

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Gram stain was done from the broth in the bottle to look for any organism. Simultaneously all the positive bottles were subcultured on blood agar and MacConkey agar plates. The plates were incubated at 37°C for 18 to 24 hours. Identification of isolated organisms and antimicrobial susceptibility was done by the VITEK 2 system.[9] Characterization of isolates was done as multidrug resistant (MDR), extensively drug resistant (XDR), and pan drug resistant (PDR). MDR was defined as acquired nonsusceptibility to at least one agent in three or more antimicrobial categories. XDR was defined as nonsusceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e., bacterial isolates remain susceptible to only one or two categories), and PDR was defined as nonsusceptibility to all agents in all antimicrobial categories.[10] Demographic, clinical, and histopathological details of the patients with positive bile culture were included. In addition, positive bile cultures were correlated with blood cultures received concurrently from the respective patient.

Statistical Analysis

Statistical analysis was performed by using the chi-squared test or Fisher's exact test as appropriate and probability levels <0.05 by the two-tailed test were considered statistically significant.

Result

A total of 226 bile samples were received in the microbiology laboratory for culture and sensitivity, 123 (54.4%) samples from male patients and 103 (45.6%) samples from female patients. Out of these, 136 (60.2%) samples were found to be culture positive. Culture positivity was similar among female (63/103 [61.2%]) and male (73/123 [59.3%]) patients. The majority of patients in both the genders were $\geq$40 years. Hypertension (33.1%) was the most common underlying disease followed by diabetes (28.7%) and cardiovascular disease (8.1%). Common manifestations presented by patients were abdominal pain (77.89%), jaundice (42.24%), and fever (41.52%). Cholangitis (acute inflammation and infection of the biliary duct system) was present in 28 (20.6%) patients. Out of 136 cholecystitis patients, 53 (39%) patients were clinically diagnosed with cholelithiasis or choledocholithiasis, 35 (25.7%) had malignancy,12 (8.8%) patients had bile duct injury, and 36 (26.4%) patients had concurrent perihepatic collection, pancreatitis, and ascites. These patients were referred patients with prior intervention (►**Table 1**). Histopathological examination revealed adenocarcinoma as the most common finding in malignant cases.

A total of 142 isolates were obtained from 136 patients with bile culture positive, as 6 (15.3%) of the samples yielded more than one organism. There was no definite pattern observed in polymicrobial infection. Among 142 isolated organisms, 120 (84.5%) were gram-negative bacilli, 19 (13.3%) were gram-positive cocci, and 3 (2.1%) were *Candida* spp. *Escherichia coli* was predominantly found in 33% bile samples followed by *Klebsiella spp.* (19%) & *Pseudomonas spp.*

Table 1 Demographic and clinical characteristics in patients with biliary tract infections ($n = 136$)

Clinical characteristics	Number (%)
Male ($n = 73$)	
<40 y	9 (6.6)
>40 y	64 (47)
Females ($n = 63$)	
<40 y	19 (13.9)
>40 y	44 (32.5)
Primary disease	
Benign	101 (74.3)
Malignant	35 (25.7)
Underlying diseases	
Hypertension	45 (33.1)
Diabetes	39 (28.7)
Cardiovascular diseases	11 (8.1)
Manifestation	
Abdominal pain	97 (71.3)
Jaundice	33 (24.3)
Fever	21 (15.4)
Route of bile collection	
Endoscopic	75 (55.1
During surgery	61 (44.9)

(18%). Among Gram positive, *Enterococcus spp*. was the most common isolate followed by *Streptococcus* and coagulase-negative *Staphylococcus*. Besides, a few isolates of *Acinetobacter* spp., *Salmonella* spp., and *Sphingomonas paucimobilis* were also obtained (►**Fig. 1**).

Further, a correlation between the age and gender of the patient with the type of isolates/pathogen from bile was also made. No significant association was seen between age and isolates from bile, whereas *Salmonella* spp. isolates were significantly high in patients younger than 40 years ($p < 0.05$). While comparing the gender with isolated bacteria, although the distribution of bacterial isolates was variable in both genders, it was not statistically significant except in the case of *Enterococcus* ($p < 0.05$). In the case of BTI with *Enterococcus*, males were significantly higher than females.

Specimens for blood culture samples were received from 40 of the patients with positive bile cultures. Out of these, only nine samples were found to be positive. However, out of these nine patients, seven blood samples showed the same organism as had been isolated from bile.

The antibiotic susceptibility profile of gram-negative isolates showed that tigecycline and aminoglycosides were the most effective drugs. *E. coli* showed 93.6 and 95.7%sensitivity to both tigecycline and amikacin. Other antibiotics that were found to be effective were gentamicin, imipenem, and cefoperazone sulbactam. The isolates were less susceptible to cephalosporins and amoxicillin clavulanate combination.

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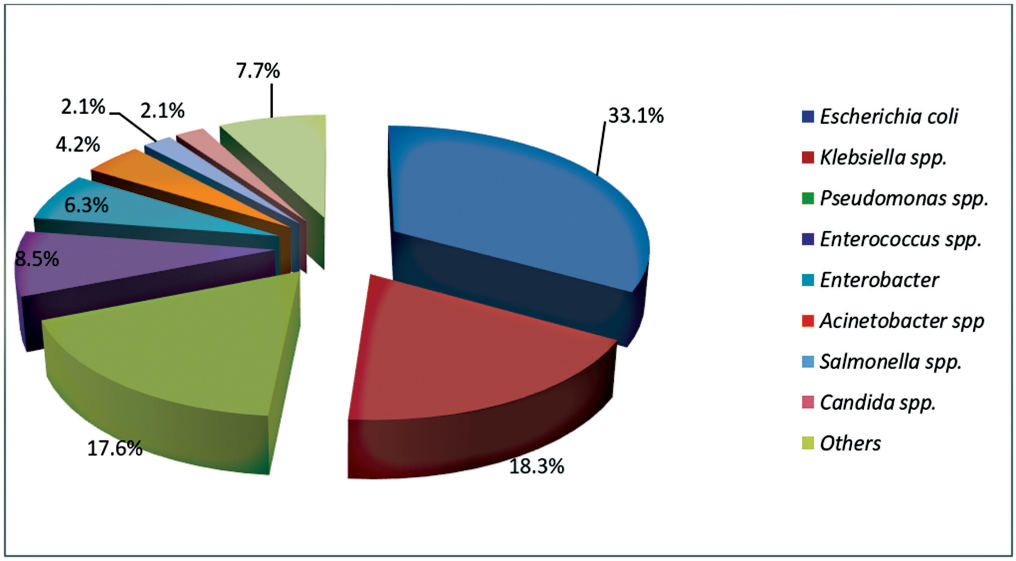


Fig. 1 Percent distribution of microorganisms isolated from bile specimens ($n = 142$).

However, *Pseudomonas* spp. and *Klebsiella* spp. showed very high resistance against cephalosporins and carbapenems (►**Fig. 2**). Among the gram-negative isolates, approximately 70% of the isolates were MDR with approximately 80 and 75% being the ESBLs and carbapenemase producers, respectively.

Gram-positive isolates (*Enterococcus*) were 100% susceptible to linezolid, whereas 25 and 16.7% of the isolates were resistant to vancomycin and teicoplanin, respectively (►**Fig. 3**).

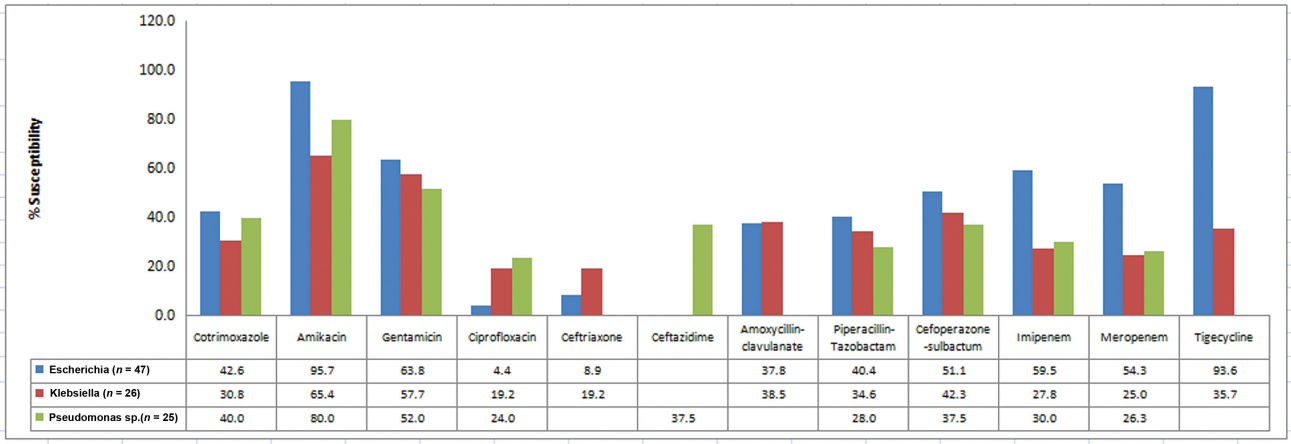
Discussion

In the present study, 136/226 (60.2%) bile samples received were found to be culture positive. Our results were in accordance with that of Shenoy et al[11] who reported a culture positivity of 56% bile samples. On the other hand, some authors have reported considerably lower bile culture positivity (23.6–26.5%).[12,13] This can be ascribed to the fact that it being a tertiary care setup most of the patients are referral patients. Additionally, with state of art in the house microbiology laboratory, the isolation rate is possibly better.

BTIs such as acute or chronic cholecystitis and cholangitis are more common in patients presenting with biliary tract obstruction due to cholelithiasis, choledocholithiasis, or malignancy.[14] We also observed that 39% of patients had a history of stones in the biliary tract and another 25.7% had malignancy.

Gender is one of the most important independent risk factors for cholelithiasis. In the present study, bile culture positivity was more in females as compared to the males, which can be correlated with the high incidence of cholelithiasis in females, although this difference was not found to be significant. Female predominance was observed in the literature.[11,14,15] In the present study, 80.2% of the patients with positive bile culture belong to the elder age groups (>40 years). Similar findings have been reported in other studies as well.[14,16]

In the present study, hypertension, diabetes, and cardiovascular diseases were the most common underlying



	Cotrimoxazole	Amikacin	Gentamicin	Ciprofloxacin	Ceftriaxone	Ceftazidime	Amoxycillin-clavulanate	Piperacillin-Tazobactam	Cefoperazone-sulbactum	Imipenem	Meropenem	Tigecycline
Escherichia (*n* = 47)	42.6	95.7	63.8	4.4	8.9		37.8	40.4	51.1	59.5	54.3	93.6
Klebsiella (*n* = 26)	30.8	65.4	57.7	19.2	19.2		38.5	34.6	42.3	27.8	25.0	35.7
Pseudomonas sp.(*n* = 25)	40.0	80.0	52.0	24.0		37.5		28.0	37.5	30.0	26.3	

Fig. 2 Antibiotic susceptibility profile of predominant gram-negative isolates.

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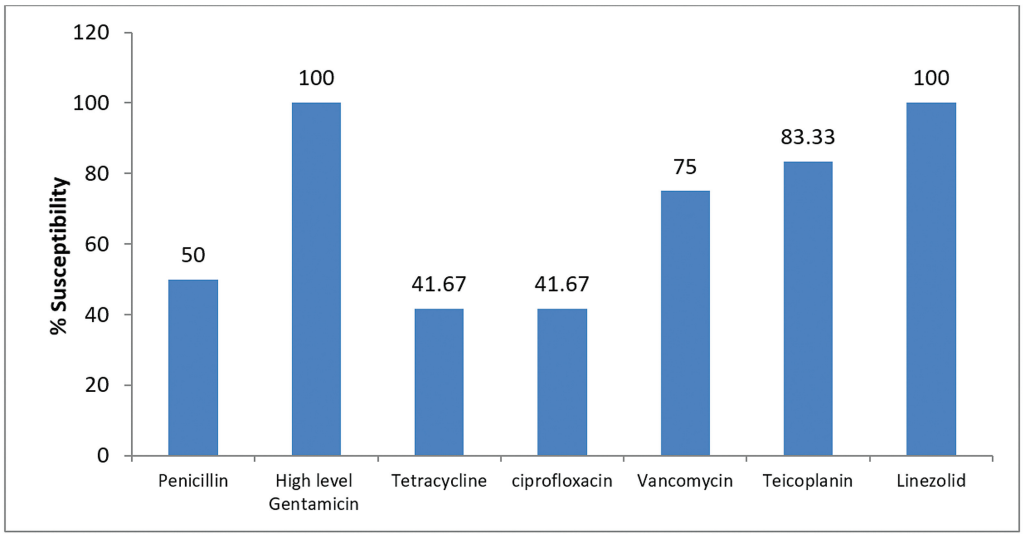

Fig. 3 Antibiotic susceptibility profile of *Enterococcus* spp. ($n = 12$).

diseases. Abdominal pain, jaundice, fever, and loss of appetite were common manifestations. Similar observations have also been reported in few other studies.[11,17]

In most cases, bile infections are caused by a single organism, but in a few cases mixed flora can be obtained. The mixed growth may be due to some antibiotic-resistant bacteria along with food habits and environmental factors. In our study, monomicrobial growth was seen in 95.6% of samples, while polymicrobial (two organisms) growths were detected in only 4.4% of the cases. Our results were in concordance with a study done by Farhana et al,[18] where a single organism was predominantly isolated from bile. Gram-negative isolates were the leading (84.5%) cause of BTIs, with a lesser number (13.3%) of gram-positive isolates, because gram-negative isolates constitute the majority of gut flora.[11] Among the gram-negative flora, consistent with previous studies, *E. coli* was the predominant isolate followed by *Klebsiella* spp. and *Pseudomonas* spp., in the present study. Among gram-positive flora, *Enterococcus* spp. remained the most common isolate as has been observed in previous studies as well.[15,16] However few other studies have reported a swift increase in the incidence of *Enterococci* in BTI, which may be due to the use of empirical antibiotic therapy against gram-negative bacteria.[19,20]

A high-level resistance against all the gram-negative isolates has been observed against β -lactam drugs including penicillin, cephalosporins, and β-lactamase inhibitor combinations. This may be correlated with the high incidence of ESBL producers among these gram-negative isolates. However, the susceptibility profile of carbapenems is variable (25–50%) in these isolates; *E. coli* is less resistant to carbapenems as compared to *K. pneumoniae* and *Pseudomonas*. Additionally, tigecycline and aminoglycosides were relatively effective against gram-negative isolates. Susceptibility to cephalosporins against gram-negative isolates was reported to be higher in comparison with aminoglycosides in a few studies, while Shahi et al observed higher resistance to cephalosporins, which corroborates with the present study.[21]

Among the gram-positive isolates, vancomycin, teicoplanin, and linezolid were the most effective drugs showing good sensitivity, which was also found in the study by Sharma et al.[22]

Only 22.5% of the patients with positive bile culture had a positive blood culture, and 77.7% of these patients had septicemia with the same organism as had been isolated from bile. Blood culture is comparatively easy to collect for isolation of etiological agents, but studies have revealed that more than 50% of patients with bactobilia were negative for blood culture,[23] probably because of lesser bacterial load in the blood of these patients.

There are no published guidelines for antibiotic treatment in bile duct infections. Therefore, this study helps determine the responsible pathogens and local antibiotic susceptibility pattern for successful treatment. The data will help in the selection of empiric antibiotics depending on the individual case. The limitations of the study include not taking data of antibiotic use before sample collection. As our hospital is a referral center, most of the patients were already on antibiotics, which can defer the actual picture of flora and antibiogram.

This study emphasizes on the flora and antibiotic profile of BTIs and enabling the clinicians to choose appropriate regimes.

In conclusion, the predominant flora was of the gram-negative organisms, exhibiting a heightened resistance to cephalosporins and carbapenems. As a result, the treatment of BTIs has become increasingly difficult. Urgent and efficacious treatment is imperative to mitigate the perils associated with such infections. Consequently, a comprehensive assessment of bacterial infection incidence and resistance patterns in individuals with BTI is instrumental in enabling health care professionals to make informed decisions in selecting the most appropriate antibiotic treatment.

Ethical Approval Statement

Ethical approval was taken for the study from the institutional ethical committee.

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Authors' Contribution

All the authors contributed equally to the article.

Conflict of Interest

None declared.

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