Original research article

Study of Microbiological Profile and Antibiotic Susceptibility Pattern in Patients with Cholangitis

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Abstract

Background: Cholangitis represents a surgical emergency which has to be managed without delay. Surgical decompression and antimicrobial therapy remain the cornerstones of this condition. However, it is important to institute the correct antimicrobial therapy considering the local resistance patterns. The resistance β-lactams has become very rampant and is mostly due to Extended Spectrum Beta lactamases (ESBL). Carbapenems are commonly used in these cases but resistance to these agents by carbapenemase enzyme production is rising. Such strains are resistant to all β-lactams and might carry plasmid-borne genes for resistance to other classes of antibiotics as well. There are a limited number of agents available for treatment of such organisms.

Methods: It was a prospective study of 100 bile samples in patients of infective biliary diseases. All the cases of cholangitis due to diverse etiology in the Department of Surgical Gastroenterology at IGIMS, Patna were studied. Samples were collected from patients of cholangitis or acute cholecystitis during Endoscopic Retrograde Cholangiopancreatography /Percutaneuous transhepatic biliary drainage/Cholecystectomy/Laparotomy.

Conclusion: The empirical therapy for cholangitis should be based upon resistance patterns in the population. Also, phenotypic detection of resistant isolates by CDT and carba NP test is reliable and helps in identifying ESBL- and carbapenemases-producers. Chromagars are sensitive for the same and can be used as screening methods.

Keywords: ESBL; Cholangitis; Bile; Carbapenemases.

Introduction

Biliary diseases form a major portion of patients presenting to surgical gastroenterology. Infections of the biliary tract (the common bile duct and gallbladder) are commonly encountered in settings of obstruction to the bile flow. The most common cause of obstruction remains gallstones leading to blockage of cystic or common bile duct, resulting in inflammation. Other causes of obstruction include tumors of the biliary tree or adjacent structures, strictures secondary to surgery or other injury, parasites (in certain geographic areas) such as *Ascaris* and *Clonorchis*[1]. Cholangitis is the inflammation of biliary tract. Inflammatory responses can be evoked by three factors-

- Mechanical inflammation- due to raised intraluminal pressure
- Chemical inflammation- due to release of lysolecithins
- Bacterial inflammation- accounts for 50-85% of patients with acute cholecystitis[2]

Clinical diagnosis of cholangitis depends upon Charcot‟s triad which includes –

- Right upper quadrant or epigastric pain
- Fever or chills or both

- Jaundice (reported in 50-70% cases)[3]

Additionally to Charcot‟s triad, signs of hypotension and altered sensorium make up the Reynolds‟ pentad, which is seen in around 14% patients with cholangitis[4]. Cholangitis can be differentiated from simple biliary colic by continuous nature of the pain. Bile is normally a sterile fluid. However, obstruction of the bile flow leads to stasis which results in growth of bacteria in bile. Increased intraluminal pressure secondary to obstruction also contributes to inflammation. Loss of antibacterial action of the bile on proximal intestines also favours bacterial growth. Bile cultures obtained from patients with cholangitis usually yield constituents of normal intestinal flora, indicating that the infection of the biliary tree is almost always endogenous and ascending. Other hypothesized routes of infection include spread via lymphatics or portal system. Most common organisms recovered are Gram negative facultative anaerobes.

Anaerobes and Gram positive cocci are rarely recovered from bile cultures[5,6].

Microbiology Of Cholangitis[5]

Bacteria	**Frequency (%)**
Gram negative organisms	
1. *Escherichia coli*	31 – 44
2. *Klebsiella* spp.	9 – 20
3. *Pseudomonas aeruginosa*	0.5 – 19
4. *Enterobacter* spp.	5 – 9
Gram positive organisms	
1. *Enterococcus* spp	3 – 34
2. *Streptococcus* spp	2 – 10
3. *Staphylococcus aureus*	0 – 4
Anaerobes	4 – 20

Emergence of antimicrobial resistance in our society is a cause of concern. Plasmids responsible for Extended Spectrum Beta Lactamase production frequently carry genes encoding resistance to other drug classes (for example, aminoglycosides). Therefore, antibiotic options in the treatment of ESBL-producing organisms are extremely limited[7]. Carbapenemase production is also emerging as an important cause of multidrug resistance. Identification of such organisms is necessary to guide the antibiotic therapy. The resistance rates are dynamic, responding to the environmental pressure applied by antimicrobial use. Hence, starting empirical antibiotic therapy with more than one antimicrobial agents and changing the therapy according to the susceptibility pattern of the pathogenic organism is suggestible. Therefore, this study is undertaken to determine the bacteriological profile and antibiogram of bile from patients with cholangitis

Objectives

1. To isolate bacteria causing cholangitis from bile samples.
2. To determine the antibiotic susceptibility pattern of the isolates.
3. To screen for ESBL (extended spectrum beta lactamase) producing bacterialstrains.

To identify carbapenemase producing organisms

Material and methods

The present study titled „Study of microbiological profile and antibiotic susceptibility pattern in patients with cholangitis‟ was carried out in the Department of Microbiology, at Indira

Gandhi Institute of Medical Sciences Patna , Bihar. Study duration of Two years. One hundred non-repetitive clinical isolates from bile samples were collected from patients admitted in Department of Surgical Gastroenterology IGIMS Patna, and studied prospectively.

Inclusion criteria
Bile samples collected from patients suspected with cholangitis.

Exclusion criteria
Samples contaminated by gut microbial flora during surgical intervention.
Samples were collected from patients with cholangitis during ERCP (Endoscopic Retrograde Cholangiopancreatography)/ PTBD (Percutaneous Transhepatic Biliary Drainage)/ CBD (Common Bile Duct) exploration. Strict precautions were taken to minimize the contamination from gut microbial flora.

Samples were put on Chocolate agar and MacConckey agar used as plating media and Brain-Heart-Infusion (BHI) broth on the day when it was received. Chococlate and MacConkey plates were examined the next day for growth. Smears were made from the suspected colonies and biochemical reactions put accordingly. Oxidase and catalase tests were performed on suspected colonies. Subcultures were done from BHI broth on the second day to Chocolate and MacConkey plates to look for the growth of fastidious and slow growing organisms. Double disk synergy test (DDST)[8]: Lawn of the test strain is exposed to the third generation cephalosporin (cefotaxime, ceftazidime, ceftraixone) and the disk of Amoxyclav (20/10 µg) is placed at a distance of 20 mm from the cephalosporin (approximately twice the distance of the inhibition zone produced by the cephalosporin when tested alone). After overnight incubation, inhibition zone of the cephalosporin disk is extended on the side nearest to the amoxyclav disk. HiCrome ESBL agar: It is recommended for selective isolation of ESBL producing Enterobacteriaceae. It is prepared by mixing HiCrome ESBL agar supplement to the ESBL agar base. *E.coli* grows as either pink or purple colonies. ESBL producing members of the *Klebsiella* group produce bluish green colonies; *Proteus*, *Morganella* and *Providencia* do not utilize any chromogen resulting in colourless to light brown colonies. Modified Hodge test: CLSI recommends this test only for *Enterobacteriaceae*. Furthermore, it is stated that sensitivity for carbapenamse types other than KPC is variable. However, it is simple to perform without any need for special media or reagents. Procedure: (1) Prepare a 0.5 McFarland standard suspension (using either direct colony suspension or growth method) of *E. coli* ATCC 25922 (the indicator organism) in broth or saline, and dilute 1:10 in saline or broth. Inoculate an MHA plate as for the routine disk diffusion procedure. Allow the plate to dry 3–10 minutes. Place the appropriate number of ertapenem or meropenem disks on the plate as noted below and shown in Figures 1 and 2. (2) Using a 10-µL loop or swab, pick 3–5 colonies of test or QC organism grown overnight on a blood agar plate and inoculate in a straight line out from the edge of the disk. The streak should be at least 20–25 mm in length. Incubation is at 37°C. Following incubation, examine the MHA plate for enhanced growth around the test or QC organism streak at the intersection of the streak and the zone of inhibition. Enhanced growth = positive for carbapenemase production. No enhanced growth = negative for carbapenemase production. Controls used are: *K. pneumoniae* ATCC BAA-1705—MHT positive and *K. pneumoniae* ATCC BAA-1706—MHT negative. Carba NP test: This is based on carbapenem hydrolysis by carbapenemase producing organisms in the presence of an indicator. This is recommended as a phenotypic confirmation test by CLSI. RAPIDEC Carb HiCrome KPC agar: This is recommended for the detection of Gram negative bacteria with resistance to carbapenems. It contains peptone, chromogenic mixture and a selective supplement. Selective supplement

inhibits the growth of yeast, Gram positive organisms and Gram negative bacilli which do not produce carbapenemases. *E.coli* produces pink to magenta colored colonies; some *C.freundii* strains can also produce similar colonies. *Klebsiella* and *Enterobacter* produce bluish green colonies. *Acinetobacter* produces smooth, colorless colonies. *Pseudomonas* produces colorless to yellowish green colonies.

Results

TABLE 1: Gender distribution of patients included in the study group

Sample	Male	Female	Total
Bile	53	47	100

Out of 100 samples, 53 (53%) were obtained from males and 47 (47%) from females. Majority of the samples were from males. Females were more likely to suffer from cholangitis secondary to choledocholithiasis and males more likely to suffer from cholangitis secondary to carcinoma of liver or biliary tree.

TABLE 2: Causative factors of cholangitis in patients included in the study group

Disease	Malignancy	Stones	Iatrogenic	Others
Percentage	38 (38%)	22(22%)	9 (9%)	31 (31%)

Out of total 100, 38(38%) patients had a malignant etiology. 22 (22%) patients were diagnosed with cholelithiasis or choledocholithiasis. 9(9%) of patients have had previous surgical intervention like cholecystectomy or placing the CBD stent. 31 (31%) of patients had other factors such as pancreatic pseudoaneurysm, strictures, choledochal cysts, blunt injury or idiopathic disease.

TABLE 3: Antibiotic sensitivity pattern of *Enterococcus* spp. (Total-4 isolates)

S/R	Amp	HLG	P	Va	Lz	Cip	Te
Sensitive	**3 (75%)**	**3 (75%)**	**2 (50%)**	**4 (100%)**	**4 (100%)**	**2 (50%)**	**4 (100%)**
Resistant	**1 (25%)**	**1 (25%)**	**2 (50%)**	**0**	**0**	**2 (50%)**	**0**

All isolates were sensitive to vancomycin, linezolide and tetracycline (100%) while only 50% isolates were sensitive to penicillin and ciprofloxacin each.

TABLE 4: Results of tests performed for detecting ESBL producers.

Test	Positive	Negative	Total
CDT (CLSI)	50 (76%)	16 (24%)	66
DDST	17 (27%)	49 (73%)	66
HiCrome agar	55 (83%)	11 (17%)	66

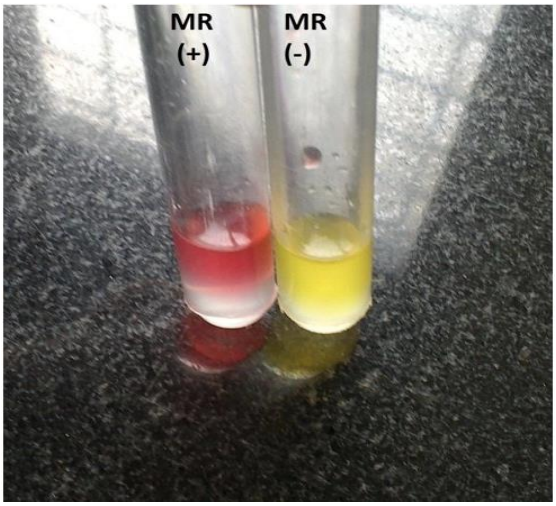
HiCrome ESBL agar identified ESBL producers 83% of times whereas CLSI method of combination disk test (CDT) identified 76% of ESBL producers. Double disk diffusion test

(DDST) detected only 27% of ESBL producers.

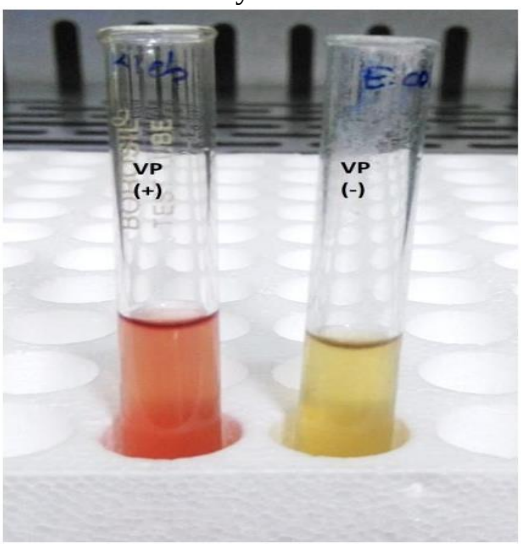
TABLE 5: Tests for detection of carbapenemase production

Test	Positive	Negative	Total
MHT	5 (25%)	15 (75%)	20
Carba NP	18 (90%)	2 (10%)	20
HiCrome agar	20 (100%)	0	20

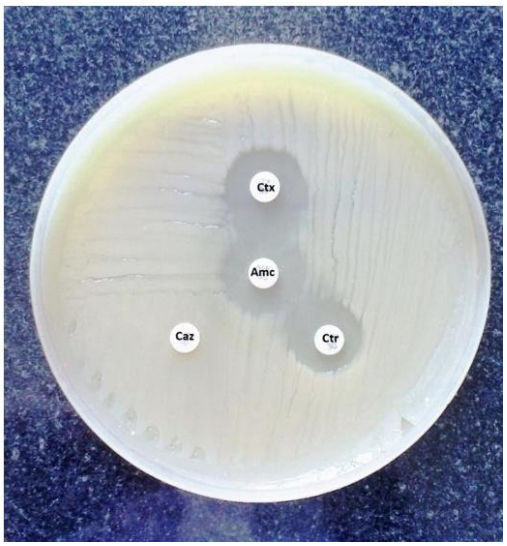
MHT identified 5% of carbapenemases producing organisms. Carba NP was positive in 90% of isolates resistant to carbapenems. Chromagar detected all the 20 isolates which were resistant to carbapenems as carbapenemases producers.



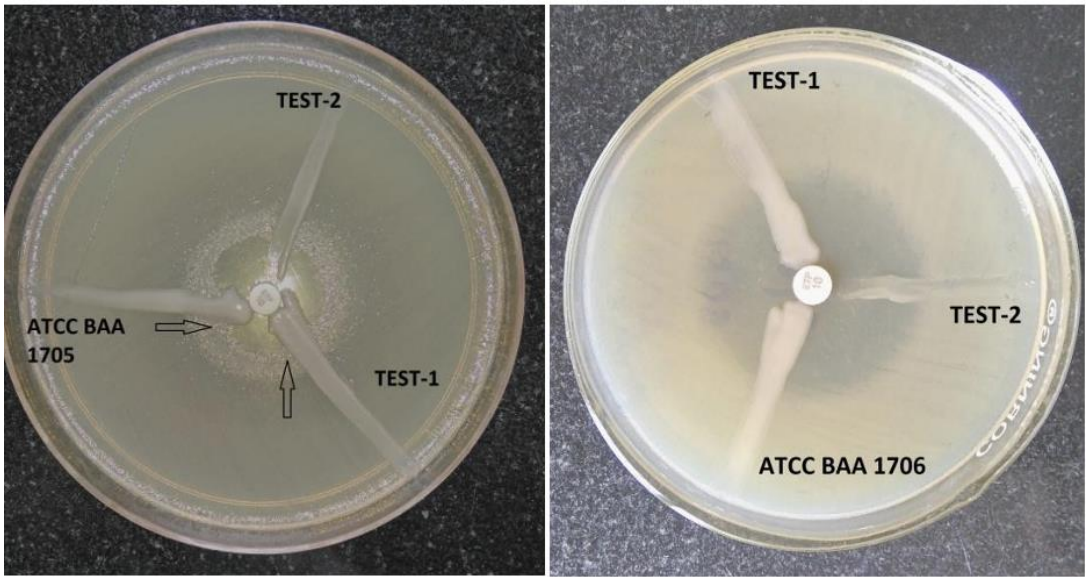
Methyl red test



Voges-Proskauer test



Double disk synergy test (DDST) for ESBL detection



Modified Hodge test (MHT) for carbapenemase detection

Discussion

Cholangitis represents one of the emergencies which have to be managed without any delay. Underlying etiologies are variable leading to the inflammation of the biliary tree. Understanding the bacteriology and antibiogram of this condition is of utmost importance for better management. Detection of resistance pattern would help in formulating empirical therapy for this condition. During processing of bile samples, direct Gram staining was performed. It had a low sensitivity, showing presence of organisms in only 23 (30%) of the 77 culture positive samples. This compares fairly with the study done by Brimsar B et al[9] who observed Gram stain and culture correlation in only 45% of samples, concluding that microscopy is unreliable in cholangitis. Predominant isolates in persent study were Gram negative bacilli belonging to *Enterobacteriaceae*. Most common isolate was *E.coli* (50%), followed by *Klebsiella* species (27%) and Gram negative non fermenters (12%). Gram positive cocci were isolated in 4% of growths. Present study compared well with observations of Chang WT et al, Shivaprakash S et al and Bapat RD et al in *E.coli* being the predominant organism and *Klebsiella* being the second most common isolate. In the study by Sung JJY[6], 85% of the patients of cholangitis responded favourably to ciprofloxacin monotherapy as

against 77% on triple therapy (Ceftazidime, Ampicillin and Metronidazole); prompting the authors to advocate ciprofloxacin monotherapy as empirical treatment for cholangitis. However, in present study, only 27% of isolates were sensitive to ciprofloxacin. It was stated by Bornman PC et al[10] that etiology such as choledocholithiasis is associated with bactibilia with *E.coli*, *Klebsiella*, *Proteus* etc. However, previous interventions significantly increase the risk of acquiring resistant bacteria such as *Pseudomonas* and *Enterobacter*. In present study, 60% of *Pseudomonas* isolates were associated with previous interventions like subtotal cholecystectomy or placing the CBD stent. Also, mezlocillin and piperacillin were said to be better in cholangitis as they are excreted in bile. However, in present study, only 50% isolates were sensitive to piperacillin-tazobactam combination. Furthermore, choosing the antimicrobial drug for biliary diseases based on their bile secretion profiles might be misleading as overt biliary obstruction prevents secretion of these drugs into bile. This can be seen in malignant biliary obstruction.

This might be the reason why such patients do not respond to antimicrobial therapy as well as those of benign lesions do[10]. In present study, majority of patients (38%) had malignant disease and hence, empirical therapy based on just biliary secretion should not be decided. Tigecycline had the best antimicrobial activity in the present study, 99% isolates being sensitive, followed by amikacin (77%) and carbapenems (76%). 94% isolates were resistant to ampicillin. Amongst Gram positive organisms, vancomycin was 100% effective. This compares fairly with the study of 209 bile samples done by Shivaprakash et al[11], wherein 92% isolates were found to be resistant to ampicillin and 90% isolates were sensitive to meropenem, 76% to amikacin and 100% to vancomycin. In present study, 66 (70%) of isolates were found to be resistant to extended- spectrum cephalosporins and 20 (21%) were found to be resistant to carbapenems. CLSI recommends combination disk method for phenotypic confirmation of ESBL production. In present study, 50 isolates were detected by CDT and 55 by chromogenic agar method. Prabha R et al[12] and Rameshkumar MR et al[13] observed similar findings. Carba NP test is another carbapenemase detection method suggested by CLSI, being superior to MHT in time required for results and ability to detect most other carbapenemases including novel types (except some-OXA types) as against MHT which detects only KPC type. Non-enterobacteriaceae can be tested by Carba NP as against MHT which can be done only on Enterobacteriaceae. In present study, 18 of 20 isolates (90%) resistant to carbapenems were positive in RAPIDEC carba NP (Biomérieux) test. This compared fairly with the study done by Österblad M et al[14], Vasoo S et al[15], and Poirel L et al[16] who observed sensitivities compared to PCR as 88%, 100% and 96% respectively. OXA-48- like enzymes were a consistent problem with this test accounting for slightly less than ideal sensitivity in some assays.

Conclusion

Bile is normally a sterile fluid. However, in cholangitis, ascending infections take place and biliary tree gets infected. Facultative anerobic bacterial infections are a major causative factor of biliary tract diseases. These organisms otherwise constitute normal intestinal flora. Majority of the bile samples obtained in this study were found to be infected with the species belonging to the *Enterobacteriaceae*.

Most of the patients included in the study were inpatients. Males were marginally more in number than females in all age groups. However, strikingly notable was the predominance of females in the only age group of 20-39 years. Patients were most likely to be middle aged or elderly. Most common etiology underlying cholangitis was found to be malignancy, followed by cholelithiasis or choledocholithiasis. Malignancy was more likely to yield culture positive

bile samples and multidrug resistant organisms.

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