e-ISSN: 0975-1556, p-ISSN:2820-2643
Available online on www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2024; 16(5); 612-617

Original Research Article

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

Rashmi Prabha[1], Raj Kishor Sharma[2], Babita Kumari[3], Vijay Kumar[4]

[1]Tutor, Department of Microbiology, PMCH, Patna
[2]M.O. CHC, Saraiya Muzaffarpur
[3]Assistant Prof. Department of Microbiology, PMCH, Patna
[4]Professor & Head, Department of Microbiology, PMCH, Patna

Received: 09-02-2024 / Revised: 03-03-2024 / Accepted: 06-04-2024
Corresponding Author: Dr. Rashmi Prabha
Conflict of interest: Nil

Abstract:

Background and Objectives: 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. To isolate bacteria causing cholangitis from bile samples. To determine the antibiotic susceptibility pattern of the isolates. To screen for ESBL (extended spectrum beta lactamase) producing bacterial strains.

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 PMCH (PMSSY), 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.

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/read), which permit unrestricted use, distribution, and reproduction in any medium, provided original work is properly credited.

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:

a) Mechanical inflammation- due to raised intraluminal pressure
b) Chemical inflammation- due to release of lysolecithins
c) Bacterial inflammation- accounts for 50-85% of patients with acute cholecystitis [2] Clinical diagnosis of cholangitis depends upon Charcot's triad which includes –

a) Right upper quadrant or epigastric pain
b) Fever or chills or both
c) 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. 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.

Objectives

- To isolate bacteria causing cholangitis from bile samples.
- To determine the antibiotic susceptibility pattern of the isolates.
- To screen for ESBL (extended spectrum beta lactamase) producing bacterial strains.
- 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, Patna medical College and Hospital Patna, Bihar. One hundred non-repetitive clinical isolates from bile samples were collected from patients admitted in Department of Surgical Gastroenterology (PMSSY).

Inclusion Criteria: Bile samples collected from patients suspected with cholangitis.

Exclusion Criteria:

Samples other than bile

Samples contaminated by gut microbial flora during surgical intervention.

Sample Collection

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. Non-repetitive 100 bile samples were processed in the Microbiology laboratory, BMC & RI, Bengaluru. The specimens were brought to the laboratory within 2 hours of collection.

Gram stain: Smears were made from all samples, heat-fixed and stained by Gram-stain. Smears were examined for the presence of pus cells and organisms.

Culture: 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.

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: Age distribution of patients included in the study group

Age in years	Male	Female	Total
0-19 years	1 (100%)	0	1 (1%)
20-39 years	8 (31%)	18 (69%)	26 (26%)
40-59 years	23 (60%)	15 (40%)	38 (38%)
60-79 years	20 (61%)	13 (39%)	33 (33%)
≥80 years	1 (50%)	1 (50%)	2 (2%)

Table 3: 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

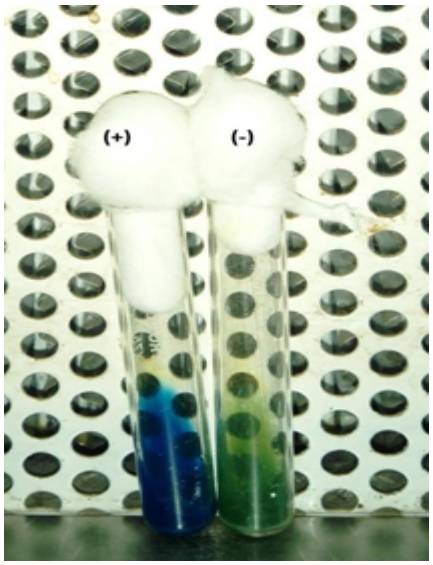
cholecystectomy or placing the CBD stent. 31 (31%) of patients had other factors such as pancreatic pseudoaneurysm, strictures, choledochal cysts, blunt injury or idiopathic disease.

Table 4: Growth on aerobic cultures from bile samples

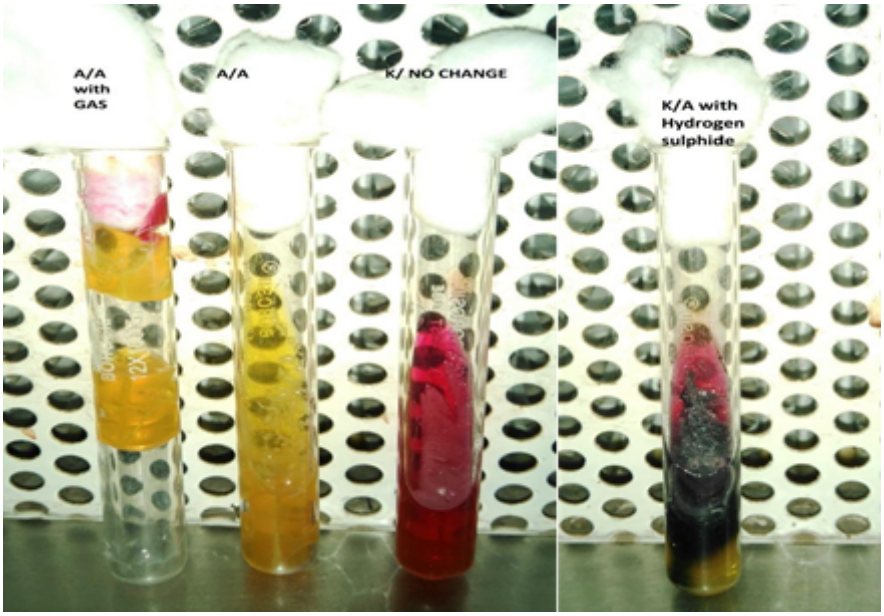
Number	Growth		No growth	Total
	Polymicrobial	Monomicrobial		
	15 (19%)	62 (81%)	23 (23%)	100

Out of 100 samples, 23 (23%) had no growth and 77 (77%) had aerobic growth. Out of these 77, 15 (19%) has polymicrobial growth and 62 (81%) had monomicrobial growth, accounting for a total of 93 bacterial isolates.

Colour plate 9: Phenylalanine deaminase test

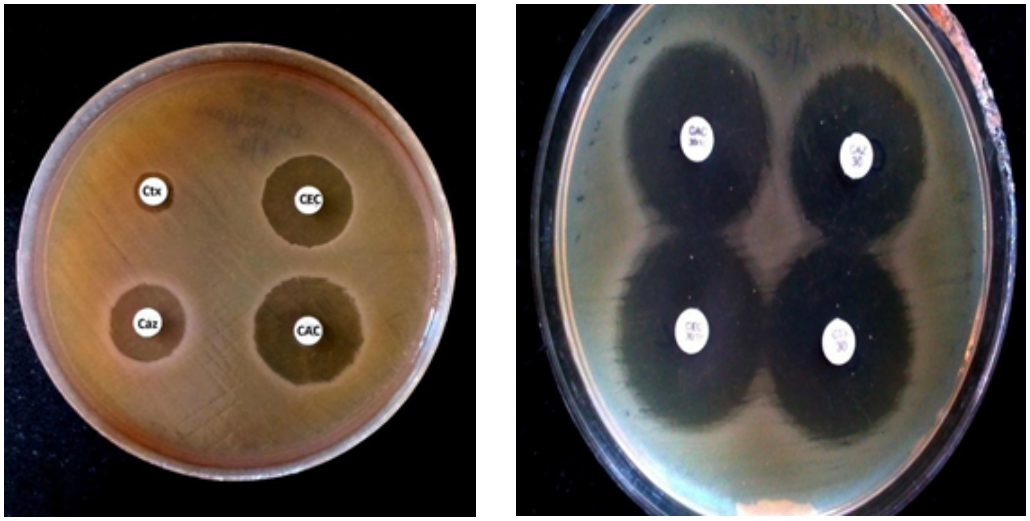


Colour plate 10: ONPG production production (PAD) test

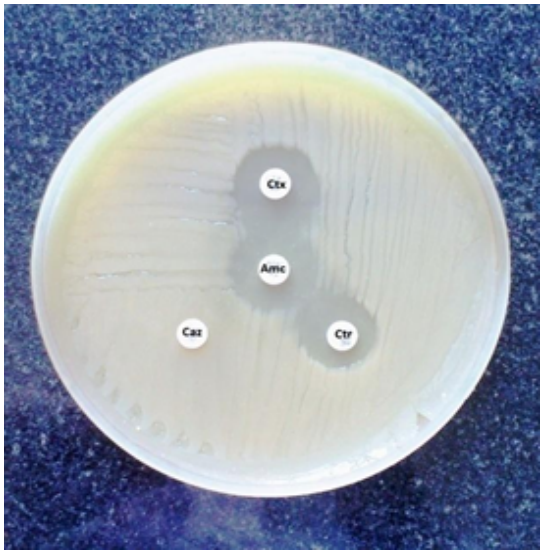


Colour plate 12: Mannitol motility medium

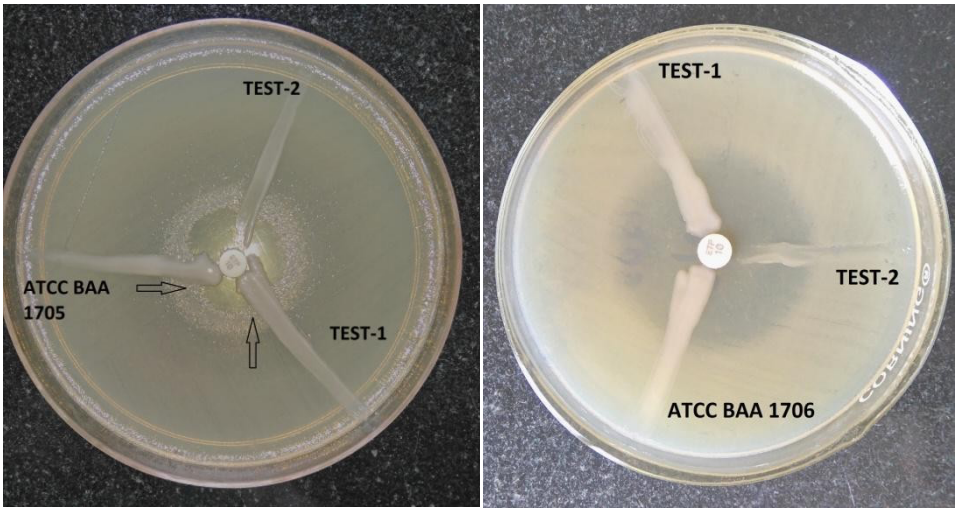
Positive for ESBL production **Negative for ESBL production**



Colour plate 16: Combination disk test (CDT) for phenotypic confirmation of ESBL production (CLSI method)



Colour plate 17: Double disk synergy test (DDST) for ESBL detection



Color plate 19: 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[14] who observed Gram stain and culture correlation in only 45% of samples, concluding that microscopy is unreliable in cholangitis.

In the present study, out of 100 bile samples, 77 (77%) showed bacterial growth.

Table 5: Bile samples showing microbial growth in various studies.

Study	Sample Size	Percentage
Shivaprakasha S et al [8] (2006)	209	61%
Lorenz R et al [9](1998)	99	73%
Chang WT et al [10] (2002)	1394	36%
Present study (2016)	100	77%

The percentage of bile samples showing growth of pathogens in present study was comparable with the study done by Shivaprakasha S et al and Lorenz R et al. Furthermore, it was observed that cholangitis owing to the presence of carcinoma of biliary tree contributed in more positive bile cultures (87%) than that in choledocholithiasis (74%).

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.

Table 6: Different isolates obtained from bile samples in various studies.

Study	Sample Size	*E.coli*	*Klebsiella spp*	*Enterococcus spp*
Chang WT [11] (2002)	1394	36%	15%	6%
Shivaprakash S [8] (2006)	209	30%	24%	12%
Bapat RD (1996)	57	30%	21%	-
Present study (2016)	100	50%	27%	4%

Present study also isolated *Acinetobacter* from 7% and *Pseudomonas* from 5% of bile samples, indicating rise of these pathogens amongst hospitalized patients. 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. Poirel L et al[13] 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. However, it still is the most cost- effective method available for rapid detection of carbapenemases.

Table 7: Sensitivities of Carba NP test in various studies.

Study	Sample Size	Sensitivity of Carba NP Test
Österblad M et al [11] (2014)	61	88%
Vasoo S et al [12] (2013)	271	100%
Poirel L et al [13] (2015)	176	96%
Present study (2016)	100	90%

Thus, it should be emphasized that determining the local resistance patterns in guiding the therapy in this population is of utmost importance. Also, rapid and newer methods should be evaluated further to establish their use in the routine laboratory practices.

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. *Escherichia coli* is a normal commensal and the most common organism associated with biliary tract infections. In our study, majority of the isolates were identified as *E.coli*. Notably, emergence of Gram negative non fermenters such as *Acinetobacter* has been observed. *Pseudomonas* was also isolated and was found to be associated with previous surgical intervention. *Enterococcus* species is also a normal commensal in intestine but is associated with multidrug resistance. Other species like *Citrobacter, Enterobacter* and *Proteus* were infrequently isolated. They are also known to inhabit the intestinal tract.

References

1. Sifri CD, Madoff LC. Infections of the Liver and Biliary System (Liver abscess, Cholangitis, Cholecystitis). In: Bennett JE, Dolin R, Blaser MJ. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 8th edition. Elsevier Saunders, Philadelphia; 2015; 1:964.
2. Greenberger NJ, Paumgartner G. Diseases of the gallbladder and bile ducts. In: Longo DL, Fauce AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J. Harrison‟s Principles of Internal Medicine, 18th edition, The McGraw-Hill Companies, Inc., 2012:2615-2628.
3. Wada K, Takada T, Kawarada Y, et al. Diagnostic criteria and severity assessment of acute cholangitis: Tokyo guidelines. J Hepatobiliary Pancreat Surg. 2007; 14:52-58.
4. Hanau LH, Steigbigel NH. Acute (ascending) cholangitis. Infect Dis Clin North Am. 2000; 14:521-546.
5. Gomi H, Solomkin JS, Takada T et al. TG13 antimicrobial therapy for acute cholangitis and cholecystitis. J Hepatobiliary Pancreat Sci. 2013; 20:60-70.
6. Sung JJ, Lyon DJ, Suen R et al. Intravenous ciprofloxacin as treatment for patients with acute suppurative cholangitis: a randomized, controlled clinical trial. J Antimicrob Chemother. 1995; 35:855-864.
7. Paterson DL, Bonomo R.A. Extended-Spectrum β-Lactamases: a Clinical Update, Clin. Microbiol. Rev. Oct 2005;18(4):657-686.
8. Apstein MD, Carey MC. Pathogenesis of cholesterol gallstones: a parsimonious hypothesi. European J Clin Investigation May 1996;26(5): 343–352.
9. Maki T. Pathogenesis of Calcium Bilirubinate Gallstone: Role of *E. coli*, P- Glucuronidase and Coagulation by Inorganic Ions, Polyelectrolytes and Agitation. Ann Surg. July 1966; 164(1):90-99.
10. Rana SS, Bhasin DK, Nanda M, Singh K. Parasitic infestations of the biliary tract.
11. Curr Gastroenterol Rep. May 2007;9(2):156-164
12. Horton J, Bilhartz L. Gallstone disease and its complications. In: Feldman M, Friedman L, Sleisenger M, editors. Sleisenger and Fordtran's gastrointestinal and liver disease: pathophysiology, diagnosis, management. Philadelphia: WB Saunders; 2002;1065–90.
13. Boey JH, Way LW. Acute cholangitis. Ann Surg 1980; 191:264–70.
14. O‟Connor MJ, Schwartz ML, McQuarrie DG, Sumer HW. Acute bacterial cholangitis: an analysis of clinical manifestation. Arch Surg 1982; 117:437–41.
15. Kadakia SC. Biliary tract emergencies: acute cholecystitis, acute cholangitis, and acute pancreatitis. Med Clin North Am 1993; 77:1015–36.