



**ORIGINAL RESEARCH PAPER**

**Gastroenterology**

**BACTERIAL ISOLATES AND ANTIBIOTIC SENSITIVITY PATTERNS IN CHOLEDOCHAL BILE COLLECTED DURING ERCP: A REPORT FROM CENTRAL INDIA.**

**KEY WORDS:** extra hepatic cholestasis, endoscopic retrograde cholangiography, cholangitis, infections

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**ABSTRACT**

Temporal shifts have been known to occur in antibiotic sensitivity patterns of organisms causing cholangitis. The aim of this prospective study conducted on 100 patients of extra hepatic biliary obstruction (EHBO) was to identify the common microorganisms cultured from bile obtained during Endoscopic retrograde cholangiography and to study their local sensitivity pattern. Sixty six patients had growth in bile culture, the maximum growths amongst all micro-organisms were of Escherichia coli (40.9%) and Pseudomonas aeruginosa (40.9%). Growth rates were significantly higher in patients with non- malignant causes of biliary obstruction than those with malignant causes. Polymixins had the highest sensitivity to cultured bacteria followed by aminoglycosides and Imipenem. Study of culture & sensitivity pattern helps in deciding empirical antibiotic therapy in patients with cholangitis and it should be based on local sensitivity patterns

**INTRODUCTION**

The continuous flushing action of bile and the bacteriostatic effects of bile salts help to keep the bile duct sterile<sup>1</sup>. However, in case of biliary obstruction, there is increase in pressure in bile duct due to bile stasis. This milieu is suitable for colonization and replication of bacteria. Eventually these bacteria spread hematogenously cause systemic infections<sup>2</sup>. In advanced biliary obstruction with sepsis and multi organ dysfunction, mortality can be as high as 10%<sup>3,4</sup>.

Studies have highlighted that blood cultures can be negative in more than half of the patients with acute cholangitis<sup>2</sup>. In suspected cholangitis, it is advisable to start empirical antibiotics before contemplating biliary drainage using endoscopic retrograde cholangiopancreatography (ERCP). Selection of antibiotic is based on numerous factors like the severity of the cholangitis, the presence of renal and hepatic dysfunction, a recent history of antimicrobial therapy, local susceptibility pattern and the biliary penetration of the antimicrobial agent<sup>5,6</sup>. Gram-negative bacilli like Escherichia coli are the most common bacterial isolates from infected bile<sup>6,7</sup>. Since Gram negative organisms are rapidly developing multi-drug resistance, the choice of appropriate empirical antimicrobial therapy has become more complicated.

The present study was conducted to study the bacterial isolates cultured from bile during ERCP and their local sensitivity pattern.

**MATERIALS AND METHODS**

This is a prospective observational study, performed at the Department of Gastroenterology of Sri Aurobindo Medical College and Postgraduate Institute, Indore, India from January 2016 to November 2017. The protocol was approved by the Institutional Ethics Committee prior to initiation of the study.

We recruited all patients of extra-hepatic biliary obstruction undergoing ERCP at our center. Informed written consent was taken from all the patients. Patients less than 18 years of age or those not consenting for participation in the study were

excluded. As per our department policy, all patients with biliary obstruction +/- suspected cholangitis are started on empirical antibiotics namely ceftriaxone in a dose of 1 gram 12 hourly. Antibiotics are escalated based on culture reports and clinical improvement.

The diagnosis of acute cholangitis was made according to Tokyo guidelines 2013 (TG13)<sup>8</sup>. Biliary obstruction was detected by dilated intra/extra-hepatic biliary system on imaging (abdominal ultrasound +/- computed tomography of the abdomen). The diagnosis of malignant biliary obstruction was based on biliary cytology, ampullary biopsy or Ultra sound guided/ Endoscopic ultrasound guided fine needle aspiration cytology.

ERCP was performed using a side viewing endoscope (TJF-150™, Olympus, Tokyo, Japan). As per our institutional policy, the scope is routinely disinfected according to the guidelines and sterilization is checked using regular smear tests and cultures every 3 months. All accessories used for ERCP were regularly sterilized using appropriate sterilizing methods. Once guide-wire cannulation is established, bile is aspirated by placing a single-use, 5.5F standard sphincterotome catheter (Ultratome™ XL, Boston scientific, Alajuela, Costa Rica) into the bile duct before the injection of contrast agent. After discarding first 2 ml of bile, approximately 2 to 6 ml of bile is collected and transferred in a sterile tube. Collected bile is transported to the microbiology laboratory under all aseptic precautions. Within 10 minutes of collection the bile sample is inoculated on blood agar and Mac conkey agar. The culture is reported at 24 and 72 hours after inoculation. In case any bacteria is isolated, antibiotic sensitivity testing is done using broth micro-dilution method.

The data recorded included age, sex, clinical symptoms, blood investigations including liver and renal biochemistry, radiological tests and history of prior antibiotic usage or attempted biliary drainage in the preceding 7 days.

Statistical analysis was done using SPSS (Statistical Package

for Social Sciences, release 20.0, standard version; SPSS Inc.) software computer package. Categorical data were presented as proportions. For qualitative data, frequency and percent distribution were calculated and for comparison between groups chi-square test was used. All *p* values were based on two-tailed tests. A *p* value < 0.05 was considered significant. One way ANOVA test was used to correlate diagnosis and growth rate.

## RESULTS

A total of 100 patients (48% males, mean age 53.53±14.65 years, range- 18 to 80 years) were included in the study. Jaundice was seen in 77(77%) patients, abdominal pain in 72 (72%) patients, fever in 44 (44%) patients, weight loss in 27 (27%) patients and altered sensorium in 3 (3%) patients.

Out of the 100 patients included in our study, 44% had cholangitis. Fifty six percent had non-malignant causes of biliary obstruction [table 1]. Fifteen (15%) of the patients had undergone previous ERCP with stenting at the time of presentation. Forty four percent of patients received empirical antibiotics prior to undergoing ERCP. There was a mortality of 5% in our study.

Out of the 100 patients, 66 patients had growth in bile culture, out of which 9 patients had growth of two microorganisms. Thus a total of 75 microbial growths were obtained. The maximum growths amongst all micro-organisms were of *Escherichia coli* (40.9%) and *Pseudomonas aeruginosa* (40.9%) as shown in table 2.

The difference in growth rates in patients having cholangitis and those not having any cholangitis (61.36% vs 69.64%) was not statistically significant (*p*=0.288). Similarly, there was no statistical difference in bacterobilia in patients who had undergone previous ERCP with stenting to those who had not undergone the same previously (60% vs. 67%, *P* =0.301). However, growth rate in patients with non-malignant causes of biliary obstruction were 70.76% as compared to 57.14% in those with malignant causes of biliary obstruction, the difference being statistically significant (*p*=0.03). We found no significant difference in growth rates in bile culture, between patients who had received antibiotics prior to ERCP and those who did not (67.92% vs. 63.83%, *P* = 0.599). Patients having bacterobilia had a mortality of 6% (4/66) during the hospital stay, as compared to only 3% (1/34) to those who did not have bacterobilia. The difference between two groups was statistically not significant. (*P*=0.173).

## Antibiotic Sensitivity

Out of 75 microbial growths obtained from 66 positive bile cultures in our study, 70 were gram negative bacterial growths, 4 were gram positive bacterial growths and 1 patient had a fungal growth.

In our study, polymyxins (Colistin – 96.97% and Polymyxin B- 85.71%) had the highest sensitivity to gram negative bacteria, followed by aminoglycosides (Amikacin- 82.09%, Gentamicin- 80.95%, and Tobramycin- 77.42%) and Imipenem (71.64%). Details have been shown in table 3.

## DISCUSSION

Acute cholangitis can be life threatening and requires urgent biliary drainage and empirical antibiotics. A recent large scale study indicated the mortality rate (30-day all-cause mortality rate) of 2.4%, 4.7%, 8.4% by TG13 severity grade 1, 2 & 3 respectively<sup>9</sup>. In patients with septic shock appropriate antimicrobial therapy should be administered within an hour<sup>10</sup>. A review of previous studies of patients undergoing biliary intervention has shown bacterobilia rates between 35-80%. Growth rates in cholangitis patients have been found to be more variable, in the range of 28-93%.<sup>5,6,11-18</sup> In our study, we found bacterobilia in 66% of patients and in patients having cholangitis it was found to be 61.36%. This high variability could be due to difference in study designs. For example, in a

study by Sahu MK et al<sup>12</sup> none of the patients were given antibiotics prior to intervention, while in our study and that of Kaya MK et al<sup>5</sup> antibiotics were given empirically to patients with cholangitis. The difference in growth rates in patients having cholangitis and those not having any cholangitis (61.36% vs 69.64%) was not statistically significant (*p*=0.288). Similar findings have been seen in the study by Kaya M et al<sup>5</sup>. However, in several other previous studies<sup>5,15,17</sup> growth rates were significantly higher in cholangitis group. The likely explanation for this finding is that antibiotics given prior to ERCP seem to reduce the probability of culturing the microorganisms causing cholangitis.

The growth rate in patients with non-malignant causes of biliary obstruction were 70.76% as compared to 57.14% in those malignant causes of biliary obstruction, the difference being statistically significant (*p*=0.03). In the study by Kaya M, et al<sup>5</sup>, there was no significant difference between the two groups in rates of bacterobilia (49% vs. 58%). In a study by Negm AA et al<sup>18</sup> also, there was no significant difference between these two groups, however it did show higher growth rates in benign strictures (84%) as compared to those with malignant strictures (76%). Cholangitis more likely to result when a bile duct that already contains bacteria become obstructed, as in most cases with choledocholithiasis. Malignant obstruction is more often complete than obstruction by a benign stricture or bile duct stone and less commonly permits reflux of bacteria from duodenal contents into the bile ducts this could be the reason of higher growth rates seen in patients with benign EHBO<sup>20</sup>.

The most common organisms cultured from bile have been *Escherichia Coli*, *Pseudomonas aeruginosa* and *Enterococci* in different studies.<sup>5,11-14,16,18, 19, 21</sup> In our study *Escherichia coli* and *Pseudomonas aeruginosa* were both equally common, followed by *Klebsiella pneumonia*

Choice of antimicrobials is crucial in management of cholangitis. In the previous studies carbapenems and aminoglycosides have been found to have highest sensitivity to gram negative bacteria cultured from bile.<sup>5,11,16,22</sup> In our study, polymyxins (Colistin – 96.97% and Polymyxin B- 85.71%) had highest sensitivity to gram negative bacteria, followed by aminoglycosides (Amikacin- 82.09%, Gentamicin- 80.95%, and Tobramycin- 77.42%) and Imipenem (71.64%). This difference from other studies has been mainly due to the fact that none of these previous quoted studies had checked for in vitro sensitivity to the Polymyxin group of antibiotics.

Interesting findings regarding carbapenem sensitivity were noted in our study. Firstly, carbapenems had a much lower sensitivity rate (Imipenem -71.64%, Meropenem-47.69%) than what was seen in previous studies<sup>5, 11, 12</sup>. Secondly, in our study Imipenem had a much higher sensitivity rate (71.64%) as compared to Meropenem (47.69%), which was not the case in any of the previous studies<sup>5, 6, 14</sup>. Although it is difficult to point out the exact reason for these findings, but one of the major contributors could be irrational and injudicious use of carbapenem antibiotics in general. These findings can also help us in the choice of empirical antibiotic therapy in future.

The limitations of our study were that anaerobic organisms were not studied in bile samples. Secondly, a larger sample size would be better suited to guide empirical therapy for cholangitis patients.

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**Table 1: Association Of Etiology Of Biliary Obstruction With Bacterial Growth**

Diagnosis.5	Growth	No-Growth	Total N== 100 (%)
CHOLEDOCHOLITHIASIS	40	16	56 (56%)
CARCINOMA GALL BLADDER	10	6	16 (16%)
PANCREATIC HEAD CARCINOMA	2	1	3 (3%)
PERIAMPULLARY CARCINOMA	5	4	9 (9%)
CHOLANGIOCARCINOMA	1	2	3 (3%)
HEPATOCELLULAR CARCINOMA	2	1	3 (3%)
BILE LEAK	2	0	2 (2%)
CHRONIC CALCIFIC PANCREATITIS	1	1	2 (2%)
PANCREATIC PSEUDOCYST	1	0	1 (1%)
PORTAL BILIOPATHY	2	1	3 (3%)
EXTRINSIC MALIGNANT COMPRESSION	0	1	1 (1%)
CBD STRICTURE	0	1	1 (1%)

(CBD= common bile duct)

**Table 2: Micro Organisms grown**

MICRO ORGANISMS	N (%)
<b>Escherichia coli</b>	27 (40.9%)
<b>Pseudomonas aeruginosa</b>	27 (40.9%)
<b>Klebsiella pneumonia</b>	13 (19.7%)
<b>Citrobacterfreundii</b>	2 (3.0%)
<b>Staphylococcus aureus</b>	2 (3.0%)
<b>Proteus mirabilis</b>	1 (1.5%)
<b>Enterococcus</b>	1 (1.5%)
<b>Diphtheroids</b>	1 (1.5%)
<b>Candida</b>	1 (1.5%)

**Table 3: Antibiotic Sensitivity pattern to Gram Negative Organisms**

Antibiotic Sensitivity	Percentage (%)
Colistin	96.97
Polymyxin B	85.71
Amikacin	82.09
Gentamicin	80.95
Tobramycin	77.42
Imipenem	71.64
Chloramphenicol	67.65
Meropenem	47.69
Doripenem	41.79
Levofloxacin	37.10
Piperacillin + Tazobactam	36.76
Ciprofloxacin	36.36
Cotrimoxazole	30.30
Ceftazidime + Clavulanic Acid	27.78
Cefepime	22.64
Cefotetan	21.05
Ticarcillin	18.75
Ticarcillin + Clavulanic Acid	15.56
Aztreonam	15.15
Cefoxitin	14.29
Cefotaxime	12.82
Ceftazidime	11.90
Ampicillin Salbactam	9.09
Cefazolin	9.09
Amoxycylav	8.11

Ampicillin	6.67
Cefuroxime	6.67

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