

# Frequency of Biliary infection and antimicrobial susceptibility pattern in patients with extra-hepatic biliary obstruction undergoing non-surgical interventions with reused accessories

## Abstract

**Introduction:** Data of the frequency of biliary infection in patients of obstructive jaundice, the microbial spectrum and antibiotic sensitivity from Asia is scant. We undertook a prospective study to evaluate the frequency and nature of biliary infection in patients with obstructive jaundice.

**Material and methods:** Bile samples collected from patients undergoing biliary drainage for Extra Hepatic Biliary Obstruction (EHBO) were cultured by standard microbiological methods and sensitivity to common antibiotics assessed as per National Committee for Clinical Laboratory Standards (NCCLS) guidelines.

**Results and discussion:** Of the total 301 EHBO patients presenting to hospital and included in study prospectively, 154 were male, 147 were female. N=208 patients underwent Endoscopic Retrograde Cholangio Pancreatography (ERCP), 93 patients underwent Percutaneous Transhepatic Biliary Drainage (PTBD). Total 212(70%) of the patients had clinical cholangitis. Bacteria were isolated in 396(78%) of the 505 bile samples. Bacteribilia was found more often in benign EHBO (85%) than in malignant (73%,  $p=0.001$ ), in patients with cholangitis (91%) than without cholangitis (58%,  $P<0.00001$ ) and in those with post procedure status (94%) than naïve (60%,  $p<0.00001$ ). Poly microbial cultures obtained in 189(50%) of positive samples were more common in cholangitis (47%) than non cholangitis group (19%,  $p<0.00001$ ) and in post biliary procedure status (49%) than in naïve (43%,  $P=0.0005$ ). Gram-negative bacilli were isolated in 485(83%) (*Escherichia coli* 50%, *Pseudomonas* 25%, *Citrobacter* 7%, *Klebsiella* 7.4%, *Proteus* 5.6% and Gram positive cocci in 17% (*Enterococcus* 83%). Gram negative bacilli were sensitive to Carbapenems, Amikacin and Piperacillin-tazobactam but show a high frequency of resistance to 3rd generation cephalosporins and quinolones. Gram positive cocci were more sensitive to Vancomycin, Teicoplanin and Amoxicillin.

**Conclusion:** Bacteribilia is seen in 78% of bile samples, bacteribilia is more common in those with clinical cholangitis, benign etiology and a history of previous biliary procedure. Poly microbial culture positivity is more common in those with cholangitis and post procedure status. Gram negative bacteria showed high frequency of resistance to 3rd generation cephalosporins and quinolones.

**Keywords:** biliary, bacterial, antibiotic, gram positive, gram negative

Volume 2 Issue 3 - 2016

Vijay Sharma,<sup>1</sup> Ghoshal U,<sup>2</sup> Baijaj SS,<sup>3</sup> Sharma R,<sup>4</sup> Choudhuri G<sup>1</sup>

<sup>1</sup>Department of Gastroenterology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, India

<sup>2</sup>Department of Microbiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, India

<sup>3</sup>Department of Interventional Radiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, India

<sup>4</sup>Department of Public Health, Regional Institute of Health, Medicine and Research, India

**Correspondence:** Vijay Sharma, Department of Gastroenterology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, 170 -B, Indira Colony, bani park, Jaipur 302016, India, Tel +9199 2801 5254, 0141401 9159, Email vijaysadashiv@gmail.com

**Received:** August 17, 2016 | **Published:** October 26, 2016

**Abbreviations:** EHBO, extra hepatic biliary obstruction; NCCLS, national committee for clinical laboratory standards; PTBD, percutaneous transhepatic biliary drainage; ERCP, endoscopic retrograde cholangio pancreatography

## Introduction

Bile is normally sterile. Bacteribilia occurs when bile flow is impaired and bacteria gain access to the biliary duct by the papilla or portal circulation. Bile duct obstruction leads sequentially to increase in intra biliary pressure, infection of bile, cholangiovenous and cholangiolymphatic reflux leading to bacteremia.<sup>1-3</sup> The bacteria described commonly in biliary tract infections include *Escherichia coli*, *Klebsiella* and *Enterococci*.<sup>4-6</sup> Cholangitis is usually more common when the biliary obstruction is partial and intermittent, as

often seen with stones in the common bile duct. Acute obstructive suppurative cholangitis a severe and fulminant form of biliary tract infections may occur when pathogenic bacteria gain access to a completely obstructed biliary system.

The presence of bacteria in the bile (Bacteribilia) of an obstructed biliary system is of concern because of the potential for progressing to cholangitis and septicemia. The frequency of bacteribilia, its chances of progressing to clinical cholangitis, the spectrum of bacteria that infect bile and their sensitivity to available antibiotics are important pieces of information that guide patient management. It helps predict cholangitis and guides the choice of appropriate antibiotics till the sensitivity reports arrive.<sup>7</sup> Further, this information helps monitor cleansing and disinfection services in an endoscopy unit and help formulate antibiotic policy for a hospital or region.

Bacterial spectrum and antibiotic sensitivity may vary in patients according to the etiology of EHBO, presence or absence of clinical cholangitis, concomitant or prior use of antibiotics and most importantly on prior intervention of biliary system. As data on biliary microbial spectrum and antibiotic sensitivity from this region is scant, we undertook a prospective study to evaluate the frequency and nature of biliary infection in patients with EHBO undergoing non-surgical biliary interventions our institute.

Infection after ERC in patients with EHBO most often results from organism already present in the obstructed system. Nosocomial pathogens may also contaminate endoscopic devices and irrigation solution.<sup>8</sup> High incidence of pseudomonas positivity in culture positive samples can be attributed to it, earlier our institute<sup>9</sup> has reported post ERCP Pseudomonas aeruginosa outbreak in 3 patients undergoing biliary drainage, isolation of similar organisms from the patient and endoscopes and accessories provided circumstantial evidence to implicate the latter as the potential source of infection. One first patient had undergone a previous procedure and he may have been infected beforehand. Post operative wound infection in biliary surgery is caused by endogenous contamination due to opening of biliary tract in patient with bactibilia, both post operative wound infection and septicemia is mostly caused by the same organism there is need for correlation of our bile culture data with post operative wound culture reports in patients in EHBO undergoing biliary surgery.<sup>10</sup>

## Patients & methods

Prospective single centre, nonrandomized study design patients presenting with clinical, biochemical and radiological evidence of biliary tract obstruction admitted for non-surgical biliary intervention were included. Bile samples were collected from patients undergoing biliary drainage ERC or PTBD and cultured by standard microbiological methods and sensitivity to common antibiotics assessed as per National Committee For Clinical Laboratory Standards (NCCLS guidelines).<sup>11</sup> Patients who declined informed consent or were unwilling to comply with study requirements and patients with refractory shock were excluded. The features essential for diagnosis of cholangitis were fever with chills, pain abdomen, leukocytosis jaundice, biochemical and radiological evidence of biliary tract obstruction.<sup>12</sup> Sepsis was defined as Systemic Inflammatory Response Syndrome (SIRS) with a proven or suspected microbial etiology. Criteria for SIRS used were as described earlier and required the presence of 2 or more of following conditions:

- i. Pyrexia (oral temperature  $>38^{\circ}\text{F}$ )
- ii. Total leukocyte count  $>11,000$  or  $<4000/\text{cu mm}$
- iii. Tachypnoea (Respiratory Rate  $>24$  breath/min)
- iv. Tachycardia (Heart rate  $>100/\text{min}$ )
- v. Evidence of systemic spread of bacteria.

Disseminated Intravascular Coagulation (DIC) was defined as bleeding disorder caused by coagulation factor as well as platelet deficiency with Fibrin Degradation Products (FDP) in blood and respiratory distress was defined as arterial oxygen saturation  $<90\text{mmHg}$  despite inspired oxygen fraction of  $>0.6\%$ .<sup>9</sup>

## Samples collection

- A. During ERC bile samples were collected after selective cannulation of bile duct with a cannula that had been sterilized

prior to the procedure by immersion in 2% Glutaraldehyde solution for at least 10minutes and then rinsed with water. Catheter was negotiated upstream to the stricture, with the help of a sterile guide wire if needed. Bile was collected in sterile disposable plastic syringe before injection of contrast agent. The syringe was capped and sent for bacteriologic studies. All patients of EHBO undergoing ERC received intravenous antibiotics half hour before the procedure as per clinical status. Patients without clinical cholangitis received Ciprofloxacin, while patients with cholangitis received, in addition, a 3rd generation cephalosporin and sometimes, Amikacin. All patients with malignant EHBO underwent biliary plastic stenting or biliary metal stenting if confirmed un-resectable, while patients with benign EHBO underwent common bile duct stone clearance, biliary stenting for stricture or biliary drainage.

- B. During PTBD bile samples were collected immediately after liver puncture, further catheter placement done according to standard procedure. Blood culture samples collected in patients with cholangitis.

## Culture methods and susceptibility tests

Bile samples were transported immediately to laboratory and inoculated directly into biphasic Brain Heart Infusion (BHI) media and in Thioglycollate broth. Whenever possible gram staining of centrifuged deposit was examined Culture were examined and subcultures made every alternate days up to 7th day, when there was turbidity or BHI media showed any growth. It was identified by standard microbiological technique.

## Blood Culture

Blood was collected using aseptic technique and inoculated either in BACTEC bottle or by manual methods using small (Robertson cooked meat broth) and big (Brain heart infusion biphasic media) bottles for up to 7days. It was examined and sub cultured on solid medium (Blood agar and MacConkey's agar) on alternate day or earlier if turbidity appeared and identified by standard microbiological method (NCCLS guidelines).<sup>11</sup>

## Results

The 301 consecutive EHBO patients studied included (154 male, median age 58, range 22- 96y) and 147 women, median age 53, range 23-83) studied, 208 underwent ERCP (111 male), 124 had benign (stone 86, stricture 32, choledochal cyst 5 and hydatid 1) and 84 had malignant disease (gallbladder 30, periampullary 21, Cholangio 19, pancreas 14). 93 patients underwent PTBD (43 male), 23 had benign (stone 5, stricture 13 and choledochal cyst 5) and 70 had malignant disease (gallbladder 40, periampullary 6, Cholangio 11, pancreas 8). 212(70%) patients had clinical cholangitis. Bile microscopy was done in 228 samples and bactibilia detected in 162(71%). Most frequently Gram negative bacilli were seen on microscopy (Table 1). Bacteria could be grown in 396(78%) of the 505 bile samples sent for aerobic culture. Gram-negative bacilli were isolated in 485(83%) and *Gram positive cocci* in 17% of samples (Table 2).

Poly microbial cultures obtained in 189(50%) of culture positive samples, were more common in patients with cholangitis (47%) than non cholangitis group (19%,  $p<0.00001$ ) and in patient who had undergone a biliary procedure (49%) than in naïve patients (43%,  $P=0.0005$ ) (Table 3).

**Table 1** Bactibilia and correlation with result of bile culture [N=228, 162 organism seen, culture same as microscopy 110(68%)]

Organism seen(column)	GNB	GPC	Both	Sterile culture
Organism cultures(row)				
GNB(n=126)	94	4	20	8
GPC(n=8)	2	2	4	-
Both(n=28)	12	-	14	2
None(n=66)	12	2	8	42

**Table 2** Bacterial isolates in culture positive samples

Bacterial species	Proportion of positive samples(%)
Gram Negative Isolates	485 (85% of total)
Escherichia Coli	50%
Pseudomonas Aeruginosa	25%
Klebsiella Pneumonie	7%
Enterobacter	3.50%
Proteus Species	6%
Acinetobacter	1%
Citrobacter Species	7%
Morgenella	<1%
Salmonella	<1 %
Gram Positive Isolates	102 (17%of total)
Enterococcus Species	84(83%)
Staphylococcus	13(13%)
Streptococcus Species	4(4%)

**Table 3** Poly-microbial flora in different groups of patients

Group	Poly microbial culture positive	Poly microbial culture negative
Benign EHBO	79(37%)	133(63%)
Malignant EHBO	110(38%)	83(62%)
ERCP group (benign EHBO)	51(41%)	73(59%)
ERCP group (malignant EHBO)	32(38%)	52(62%)
PTBD group (benign EHBO)	28(32%)	60(68%)
PTBD group (malignant EHBO)	78(37%)	131(63%)
Cholangitis group	159(47%)	176(53%)
No Cholangitis group	33(19%)	137(81%)
Cholangitis group (ERCP)	64(48%)	73(52%)
No Cholangitis (ERCP)	18(26%)	50(74%)
Cholangitis group (PTBD)	92(47%)	103(53%)
No Cholangitis (PTBD)	15(15%)	87(85%)

Table Continued....

Group	Poly microbial culture positive	Poly microbial culture negative
Naïve patient	96(43%)	128(57%)
Post procedure status	139(49%)	142(51%)
Naïve patients (ERCP)	39(31%)	86(69%)
Post procedure status (ERCP)	46(55%)	37(45%)
Naïve patients (PTBD)	57(60%)	42(40%)
Post procedure (PTBD)	93(47%)	105(53%)
High block (malignant EHBO)	63(58%)	45(42%)
Low block (malignant EHBO)	45(48%)	62(52%)

## Discussion

In previous studies factors associated with bactibilia were age >65years, jaundice, benign etiology like choledocholithiasis and benign biliary stricture, previous biliary procedure, acute cholecystitis and previous Cholangitis.<sup>13</sup> Another study by Targarona EM et al.<sup>14</sup> demonstrated that presence of cholangitis, benign etiology of EHBO like choledocholithiasis/benign biliary stricture, and history of previous biliary procedure were associated with positive bile cultures in 50% of cases. In comparison<sup>15</sup> none of these factors was present, only 15% had bactibilia.

In study by Leung JW et al.<sup>16</sup> most common organism found in infected bile were gram negative enteric aerobes like E coli, Pseudomonas, Klebsiella and Proteus, similar results were found in our study which revealed Escherichia coli (50%), Pseudomonas(25%), Klebsiella (7%), Citrobacter (7%) and Proteus(6%) but bacterial spectrum in malignant obstructive jaundice is different compared to another report from India<sup>17</sup> in which E. coli (36.6%), Klebsiella pneumonie (18.3%), Pseudomonas aeruginosa (8.3%), Proteus vulgaris (8.3%) and Coagulase negative staphylococci (8.3%). Gram-positive isolates are increasing in frequency; our study revealed 17% of total isolates were GPC. Blood culture was positive in 39/223(18%) cases, in only 12 samples (31%) organism isolated were same as that of bile culture 12(31%). Coagulase negative staphylococcus (n=14) was isolated in maximum number of cases, followed by E. coli (n=7), Pseudomonas (n=6), Acinetobacter (n=4), Enterobacter (n=3), Enterococcus (n=2) and Proteus (n=1). Organism seen on bile microscopy are generally the organism grown on culture, this observation was corroborated by our study.<sup>16</sup> One study from India has confirmed similar findings of bacterial spectrum and antibiotic sensitivity, though number of patient was smaller than our study.<sup>17</sup>

Gram-negative isolates were most sensitive to Carbapenem, Amikacin, Piperacillin-tazobactam and Gram-positive isolates were sensitive to Amoxicillin, Vancomycin, Teicoplanin and Tetracycline in descending order (Table 4). Bile culture was positive more often in benign EHBO patients, in patients with cholangitis in patients who had undergone a biliary procedure (Table 5).

We found higher frequency of Proteus and Enterococcus in bile of patients with cholangitis and higher frequency of Pseudomonas grown in bile samples of patients undergoing ERCP without cholangitis. Enterococcus was more frequently grown in high degree block and with previous history of biliary procedure, while higher frequency of E coli, Klebsiella and Enterococcus in malignant EHBO patients with higher (type 3 and 4) (Table 6).

**Table 4** Antibiotic sensitivity of bacterial isolates (sensitive isolates in %)

Bacterial species/ Antibiotic	E.coli (N=242)	Pseudomonas (N=121)	Klebsiella (N=36)	Enterobacter (N=17)	Citrobacter (N=34)	Proteus (N=27)	Acinetobacter (N=5)	Entero coccus+ strept. spe(N=88)	Staph. species (N=13)
Amoxicillin	5%	-	8%	-	6%	-	20%	76%	46%
Gentamycin	20%	26%	11%	6%	24%	4%	40%	21%	77%
Amikacin	55%	50%	53%	24%	71%	33%	80%	-	62%
Ceftriaxone	16%	5%	14%	12%	21%	37%	60%	-	-
Ceftazidime	-	49%	14%	-	20%	11%	20%	-	-
Piperacillin- tazobactam	43%	76%	28%	88%	53%	52%	60%	-	-
Carbapenam	79%	51%	70%	71%	100%	56%	40%	-	-
Cotrimoxazole	13%	-	-	-	-	22%	-	-	-
Fluoroquinolones	20%	31%	19%	59%	24%	-	60%	29%	62%
Tetracycline	-	22%	-	-	18%	-	-	71%	54%
Oxacillin	-	-	-	-	-	-	-	-	-
Vancomycin	-	-	-	-	-	-	-	69%	100%
Teicoplanin	-	-	-	-	-	-	-	75%	-
Erythromycin	-	-	-	-	-	-	-	17%	46%
Cefazolin	-	-	-	-	-	-	-	-	46%

**Table 5** Incidence of bacteribilia in different groups of patients (total number, n=505)

Different groups compared	Positive	Negative
ERCP group	172(83%)	36(17%)
PTBD group	224(75%)	73(25%)
Benign EHBO	181(85%)	31(15%)
Malignant EHBO	215(73%)	78(17%)
ERCP group(Benign EHBO )	103(83%)	31(17%)
ERCP group(malignant EHBO)	69(82%)	15(18%)
PTBD group(benign EHBO)	78(89%)	10(11%)
PTBD group (malignant EHBO)	146(70%)	63(30%)
Cholangitis group	298(89%)	37(11%)
No Cholangitis group	98(58%)	72(42%)
Cholangitis group(ERCP)	130(93%)	10(7%)
No Cholangitis group(ERCP)	41(60%)	27(40%)
Cholangitis group (PTBD)	175(90%)	20(10%)
No Cholangitis (PTBD)	57(58%)	45(42%)
Naïve patients	134(60%)	90(40%)
Post procedure status	262(94%)	19(6%)
Naïve patients (ERCP)	91(73%)	34(27%)
Post procedure status (ERCP)	78(94%)	5(6%)
Naïve patients (PTBD)	42(42%)	57(58%)
Post procedure (PTBD)	183(92%)	14(8%)
High block (malignant EHBO) n=164	108(66%)	56(44%)
Low block (malignant EHBO) n=139	107(77%)	32(27%)

**Table 6** Comparison of biliary bacterial spectrum in different groups of patient

Bacterial species/ Groups of patients	E. coli	Pseu- domo- nas	Kleb- siella	Citro bacter	Ente- robac- ter	Pro- teus	Acine- tobac- ter	Morge- nella	Sta- ph.	Strept.	Entero- cocci
ERCP gr (total n=172)	52%	35%	9%	9%	1%	10%	1%	1%	3%	2%	18%
PTBD gr (total n=224)	67%	27%	9%	8%	7%	4%	2%	-	4%	3%	21%
ERCP(Benign EHBO) n=103	56%	35%	12%	15%	1%	11%	1%	2%	4%	3%	17%
ERCP(Malignant EHBO) n=68	45%	39%	6%	-	1%	9%	-	-	3%	1%	20%
PTBD(benign) n=78	72%	21%	4%	6%	4%	9%	1%	-	-	1%	17%
PTBD(malignant) n=146	66%	30%	12%	10%	3%	8%	1%	-	5%	3%	23%
Cholangitis(ERCP gr) n=130	50%	33%	10%	14%	2%	12%	1%	1%	2%	2%	22%
No Cholangitis (ERCP gr) n=41	56%	41%	7%	12%	-	5%	-	2%	7%	5%	10%
PTBD gr (Cholangitis) n=179	62%	30%	9%	9%	5%	8%	2%	-	4%	3%	21%
PTBD gr (no Cholangitis) n=57	76%	16%	9%	4%	1%	1%	-	-	3%	-	16%
ERCP gr (naive ) n=92	49%	29%	9%	11%	2%	10%	1%	2%	2%	3%	15%
ERCP gr(post procedure) n=78	62%	41%	9%	14%	-	10%	-	-	5%	1%	24%

## Conclusion

Bacteribilia is seen in 78% of bile samples, it is more common in those with clinical cholangitis, benign etiology and a history of previous biliary procedure. Poly microbial culture positivity is more common in those with cholangitis and previous history of biliary procedure. Gram negative bacilli are sensitive to Carbapenems, Amikacin and Piperacillin-tazobactam. High frequency of resistance to 3rd generation cephalosporins and quinolones was found. Streptococcus species were sensitive to Teicoplanin, Vancomycin and Amoxicillin while Staphylococcus was sensitive to Vancomycin, Gentamycin, Amikacin and Fluoro quinolones in decreasing frequency.

## Acknowledgements

None.

## Conflict of interest

Author declares that there is no conflict of interest.

## References

- Carpenter HA. Bacterial and parasitic cholangitis. *Mayo Clin Proc.* 1998;73(5):473–478.
- Pitt HA, Cameron JL. Acute Cholangitis. In: Way LW, Pelligrini CA, editors. *Surgery of gall bladder & bile ducts.* Pennsylvania, USA: WB Saunders; 1987. p. 295–313.
- Lipsett PA, Pitt HA. Acute cholangitis. *Surg Clin North Am.* 1990;70(6):1297–1312.
- England DM, Rosenblatt JE. Anaerobes in human biliary tracts. *J Clin Microbiol.* 1977;6(5):494–498.
- Finegold SM. *Anaerobic bacteria in human disease.* New York, USA: Academic Press Inc; 1977.
- Nielsen ML, Justesen T. Anaerobic and aerobic bacteriological studies in biliary tract disease. *Scand J Gastroenterol.* 1976;11(5):437–446.
- Hazel SJV, Speelman P, Tytgat GN, et al. Role of antibiotics in the treatment and prevention of acute and recurrent cholangitis. *Clin Infect Dis.* 1994;19(2):279–286.
- Wells GR, Taylor EW, Lindsay G, et al. Relationship between bile colonization, high-risk factors and postoperative sepsis in patients undergoing biliary tract operations while receiving a prophylactic antibiotic. West of Scotland Surgical Infection Study Group. *Br J Surg.* 1989;76(4):374–377.
- Ranjan P, Das K, Ayyagiri A, et al. A report of post-ERCP Pseudomonas aeruginosa infection outbreak. *Indian J Gastroenterol.* 2005;24(3):131–132.
- Lewis RT, Goodall RG, Marien B, et al. Biliary bacteria, antibiotic use and wound infection in surgery of gallbladder and common bile duct. *Arch Surg.* 1987;122(1):44–47.
- Kasper DL, Braunwald E, Hauser S, et al. Harrison's Principles of Internal Medicine. 16th ed. *Neurology.* 2005;64(8):1488–1489.
- Keighley MR, Flinn R, Williams JA. Multivariate analysis of clinical and operative findings associated with biliary sepsis. *Br J Surg.* 1976;63(7):528–531.
- Thompson JE, Bennion RS, Doty JE, et al. Predictive factors for bacteribilia in acute cholecystitis. *Arch Surg.* 1990;125(2):261–264.

14. Targarona EM, Garau J, Ramos CM, et al. Single dose antibiotic prophylaxis in patients at high risk of biliary infection in biliary surgery: a prospective and randomized study comparing cefonicid and mezlocillin. *Surgery*. 1990;107(3):327–334.
15. Reiss R, Eliashiv A, Deutsch AA. Septic complication of bile culture in 800 consecutive cholecystectomies. *World J Surg*. 1982;6(2):195–199.
16. Leung JW, Ling TK, Chan RC, et al. Antibiotics, biliary sepsis, and bile duct stones. *Gastrointest Endosc*. 1994;40(6):716–721.
17. Neve R, Biswas S, Dhir V, et al. Bile culture and antibiotic sensitivity in malignant obstructive jaundice. *Indian J Gastroenterol*. 2003;22(1):16–18.