

Multidrug Resistant Bacteria Causing Biliary Tract Infection: A Tertiary Care Centre Study from North India

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Abstract

Higher morbidity and mortality is seen with bacteraemia caused by Biliary tract infection. Due to the presence of biliary sphincter, bile remains sterile despite presence of high concentration of bacteria in duodenum. Increased ductal pressure due to obstruction in biliary tree, can result in biliary stasis and bacterial multiplication resulting in cholangitis. 16% to 85% of bacteriobilia have been reported in various studies worldwide in patients of biliary tract infection. Govind Ballabh Pant institute of Postgraduate Medical Education & Research (GIPMER) is a super speciality hospital providing care to patients from all over India. This study was performed to identify the bacteria causing Biliary tract infection and study their resistant pattern. A total of 229 intraoperative bile aspirates from gastrointestinal surgery department were received in microbiology department of GIPMER and analysed. Identification and antimicrobial susceptibility testing was performed as per standard techniques. Bacterial growth was seen in 166(72.48%) out of 229 patients. Polymicrobial cultures 27(16.27%) were less frequent in comparison with Monomicrobial growth 139(83.73%). Commonest isolate was *Escherichia coli* (47.94%) followed by *Klebsiella pneumonia* (17.53%), *Pseudomonas aeruginosa* (16.49%), and *Enterococcus spp.* (11.34%). High resistance was observed against routinely used antibiotics by Gram negative bacteria, such as ciprofloxacin, ceftriaxone, piperacillin/tazobactam, and low resistance was observed against carbapenems and colistin. The study shows that analysis of bile can be valuable in preparing a better treatment strategy in biliary tract infection. Rapid development of multidrug resistance in gram-negative bacteria causing biliary tract infections remains the cause of concern.

Keywords: Bactobilia, biliary tract infection, multidrug resistant bacteria, bile.

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INTRODUCTION

Gall bladder disease with its complications dominates disorders of biliary tract. Higher morbidity and mortality is seen with bacteraemia caused by Biliary tract infection. Due to the presence of biliary sphincter, bile remains sterile despite presence of high concentration of bacteria in duodenum. Increased ductal pressure due to obstruction in biliary tree, can result in biliary stasis and bacterial multiplication resulting in cholangitis.

Acute cholangitis is defined as inflammation in biliary tree due to bacterial proliferation or non-bacterial infection, caused by biliary obstruction [1].

Complete obstruction of common bile duct alongwith proliferation of pathogenic bacteria may lead to severe form of biliary tract infection i.e. Acute obstructive suppurative cholangitis [2]. The organisms of Enterobacteriaceae family are most common causative agents as they can ascend from the gastrointestinal tract [3].

Invasion of biliary tract by bacteria may occur by hematogenous route via hepatic portal vein or by bacteria ascending from the duodenum. The percentage of bactobilia increases from 3% to 30% in patients with gallstones and common bile duct stones, respectively, whereas in healthy persons excretion of bile on daily basis leads to flushing of bacteria entering the biliary tract [4].

16% to 85% of bacteriobilia have been reported in various studies worldwide in patients of biliary tract infection. Govind Ballabh Pant Institute of Post Graduate Medical Education & Research (GIPMER) is a super speciality hospital providing care to patients from all over India. The present study was performed to determine the incidence of biliary tract infection, to identify the common bacteria causing infection and study their antibiotic resistance pattern.

MATERIAL & METHODS

A prospective observational study was carried out from June 2017 to May 2018. A total of 229 intraoperative bile aspirates from gastrointestinal surgery department were received in microbiology laboratory of GIPMER and analysed. Gram staining of the sample was done. The bile samples were inoculated on 5% Sheep Blood agar and MacConkey's agar. The

culture plates were incubated overnight for 16 to 18 hrs at 37 degrees. The samples were inoculated into glucose broth for enhancing growth of bacteria. Subculture was done from broth if no growth was observed after 18-24 hrs. Bacterial identification and antimicrobial susceptibility testing was performed by VITEK-2 Compact Automated Systems and Kirby Bauer disc diffusion method as per Clinical and Laboratory Standards Institute (CLSI) guidelines.

RESULTS

Total of 229 bile samples were received in microbiology laboratory of GIPMER during the study period. The male: female ratio was 1:1.72 in the study population and the mean age was 48.24yrs. Female predominance (63.31%) was observed over males (36.68%).

Table-1: Distribution of patients as per age groups

Age group	No. of patients	Male	Female
0-20 years	3	0	03
21-40years	63	14	49
41-60years	122	51	71
Above 60 years	41	19	22
Grand Total	229	84	145

Among 229 patients, 72 had malignant etiology (Carcinoma gallbladder, Carcinoma Head Of Pancreas, Periampullary Carcinoma etc) and 157 were of benign etiology (gallbladder stone, cholecystitis, benign biliary stricture, choledochal cyst etc). Positive bacterial growth was observed in 166(72.48%) out of 229 bile cultures. Sixty three bile samples were sterile. Polymicrobial cultures 27(16.27%) were less frequent in comparison with Monomicrobial growth 139(83.73%). One hundred ninety four bacterial strains

were isolated from 166 positive bile cultures. One hundred forty four samples showed growth of gram negative bacteria, 13 samples grew both gram positive and gram negative organism whereas 9 samples showed growth of only gram positive organism. Female predominance was observed over males (Table-1). Commonest isolate was *Escherichia coli* (47.94%) followed by *Klebsiella pneumoniae* (17.53%), *Pseudomonas aeruginosa* (16.49%), and *Enterococcus spp* (11.34%) (Table-2).

Table-2: Frequency of isolation of microorganism from bile

Organism isolated	Number of Isolates (n=194)	Percentage
<i>Escherichia coli</i>	93	47.94%
<i>Klebsiella pneumoniae</i>	34	17.53%
<i>Pseudomonas aeruginosa</i>	32	16.49%
<i>Enterococcus spp.</i>	22	11.34%
<i>Acinetobacter spp.</i>	5	2.58%
<i>Enterobacter spp.</i>	5	2.58%
<i>Citrobacter freundii</i>	2	1.03%
<i>Proteus mirabilis</i>	1	0.52%

Table-3: Antibiotic resistance pattern of Gram negative bacteria

Antibiotic	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>
Ampicillin	95.70%	97.10%	NT
Amoxicillin-clavulanate	67.70%	50.00%	NT
Amikacin	17.20%	32.40%	28.13%
Gentamicin	33.30%	38.20%	34.38%
Cefotaxime	91.40%	82.40%	87.50%
Cefuroxime	91.40%	82.40%	87.50%
Ceftriaxone	91.40%	82.40%	87.50%
Cefepime	91.40%	82.40%	87.50%
Ceftazidime	91.40%	79.40%	84.38%
Cefoperazone-sulbactam	47.30%	52.90%	71.88%
Ciprofloxacin	89.20%	61.80%	65.63%
Levofloxacin	84.90%	55.90%	65.63%
Ofloxacin	84.90%	55.90%	NT
Tobramycin	47.30%	44.10%	21.88%
Netilmicin	51.60%	50.00%	34.38%
Ticarcillin-clavulanate	82.80%	82.40%	87.50%
Piperacillin-tazobactam	49.50%	44.10%	50.00%
Imipenem	26.90%	35.30%	15.63%
Meropenem	32.30%	41.20%	37.50%
Ertapenem	31.20%	38.20%	NT
Colistin	0.00%	0.00%	0.00%
Tigecycline	0.00%	5.90%	NT

NT – Not tested

Table-4: Antibiotic susceptibility of *Enterococcus spp*

Antibiotic	Sensitive	Resistant
Ampicillin	63.63%	36.36%
High Level Gentamicin (120µg)	86.36%	13.63%
Vancomycin	100%	0%
Teicoplanin	100%	0%
Linezolid	100%	0%

DISCUSSION

Gallbladder disease, commonly manifests as gallstones. Patients often remain undiagnosed because cholelithiasis is usually asymptomatic and often the patient presents with complications viz. acute and chronic cholecystitis. Gallbladder diseases in Indians are known to occur earlier, especially, in middle aged fat (obese) multiparous women. North India is the leading population in India where gallbladder disease is seven times more common as compared to other parts of the country. The patients are treated repeatedly with ineffective multiple courses of antibiotics and the median period is nearly three years before they present for surgical procedures [5]. Infected bile in biliary tract infection acts as a risk factor for various complications such as wound infection, sepsis and bacteremia.

The male: female ratio was 1:1.72 in the study population and the mean age was 48.24yrs. Similar observation were found by various studies –Ahmad *et al.*, [6] and Manan *et al.*, [7] who recorded male to female ratio of 1: 3.85.

Bacteriobilia is a commonly seen in patients with risk factors, such as biliary obstruction due to

acute cholecystitis or common bile duct stones, and nonfunctioning gallbladders [8, 9]. In our study, among 229 patients, 157 were of benign etiology (gall bladder stone, cholecystitis, benign biliary stricture, choledochal cyst etc) and 72 had malignant etiology (Carcinoma gallbladder, Carcinoma Head of pancreas, periampullary carcinoma etc). This is similar to Shenoy *et al.*, [1] who reported 36.8% malignant causes in his study.

In our study, 88.6% of the isolates were Gram negative enteric bacteria, with *Escherichia coli* (47.94%), *Klebsiella pneumoniae* (17.53%), and *Pseudomonas aeruginosa* (16.49%), being the most common organisms. In previous studies, 70–78% of positive bile cultures yielded Gram negative enteric aerobes in which *E. coli* and *Klebsiella spp* were the most common [1, 6, 10-13]. This study was conducted in a tertiary care setup with an in house microbiology laboratory, which may have led to better isolation of the pathogens without any delay in transport. We also recovered 5 isolates of *Acinetobacter* species and *Enterobacter spp.* each.

Study done by Ghahramani *et al.*, showed that prolonged bile duct obstruction leads to an impaired intestinal wall barrier resulting in easy colonization of bile by gram negative enteric bacteria, especially *E.coli*

[14]. Hence, most common bacteria causing bacteriobilia was reported as *E.coli* in many studies including our study (Table-5).

Table-5: Various studies showing *E.coli* as most common organism isolated from bile

Study	Year	Sample size	Prevalence of Infection	Commonest organism
Neve <i>et al.</i> , [11]	2001	65	52.3 %	<i>E.coli</i>
Ballal <i>et al.</i> , [12]	2001	125	56.8 %	<i>E.coli</i>
Capoor <i>et al.</i> , [15]	2005-2007	104	35.6 %	<i>E.coli</i>
Ahmad <i>et al.</i> , [6]	2013	268	58.58 %	<i>E.coli</i>
Shenoy <i>et al.</i> , [1]	2013	345	54 %	<i>E.coli</i>
Katyal <i>et al.</i> , [13]	2015	110	31.81 %	<i>E.coli</i>
Present study	2017	229	72.48 %	<i>E.coli</i>

Manan *et al.*, reported a potential role for *E.coli*'s glucuronidase enzymatic activity in formation of calcium bilirubinate gallstone [7].

Emphasis should be on developing local antibiotic sensitivity data since significant number of cases of cholangitis show polymicrobial aetiology and high rate of antibiotic resistance. The data helps to select the empirical antibiotics depending on the individual cases [1].

Polymicrobial cultures 27(16.27%) were less frequent in comparison with Monomicrobial growth 139(83.73%) in our study. Shenoy *et al.*, reported 31.5% polymicrobial infection in their study. The association of polymicrobial infection with obstruction due to cholelithiasis or previous manipulation of the biliary tract has been widely reported by various authors [16-18]. The importance lie in the fact that the stone may be acting as a source of infection with the bacteria trapped in the center, hence, in such cases it becomes imperative to remove stone prior to starting antibiotic therapy. Also, formation of calculi causes stasis, which promotes chronic infection leading to increase in conversion of primary bile acids to secondary bile acids, which in turn causes constant irritation of the biliary tree and increased chances of infection [15].

In our study, resistance for ampicillin was 95-97% Cephalosporins showed a very high resistance of 79-91%. However, piperacillin-tazobactam, imipenem and meropenem had an efficacy ranging from 50% to 75%. Aminoglycosides (gentamicin and amikacin) showed less resistance from 26-38% and quinolones (ciprofloxacin and ofloxacin) showed high resistance rate ranging from 55% to 90% (Table-3). The synergistic action of aminoglycoside and ampicillin provides good coverage against most organisms. Due to high rate of resistance as seen in our study, ampicillin should not be a choice of empirical antibiotic in our setup. Shenoy *et al.*, [1] suggests that the ideal antibiotics in case of biliary tract infection would be those, which are excreted in the bile such as third-generation cephalosporins, fluoroquinolones,

ureidopenicillins (mezlocillin and piperacillin), and carbapenems. Various gram negative bacteria causing polymicrobial infection justifies the selection of these broad spectrum antibiotics.

In a study by Manan *et al.*, [7], *E. coli* showed 78.57% sensitivity to Cefuroxime, 75.00% to Ceftriaxone and resistance to Amoxicillin (60.71%) and Ciprofloxacin 42.86%. *Klebsiella* showed 72.22% sensitivity to Ciprofloxacin and 55.56% resistance to Amoxicillin. Study done by Gomes PRL *et al.*, [19], showed coliforms were 100% sensitive to imipenem, 95% to gentamicin and amikacin, 81% to amoxycylav and piperacillin. Cefotaxime (100%) and ceftriaxone (95%) were more effective against coliforms than cefuroxime (86%).

In the present study, 22 *Enterococcus spp.* were 100% sensitive to Teicoplanin, Vancomycin and Linezolid (Table-4). This finding is similar to Shenoy *et al.*, who reported 23 *Enterococcus spp.* isolates all sensitive to Teicoplanin and Vancomycin.

Bornman *et al.*, [20] suggests that as long as there is biliary obstruction, the concentration and excretion of the antibiotic will not be accurate. Capoor *et al.*, [15] mentions firstly the patients seek medical care usually from local medical practitioners and receive repeated, suboptimal and ineffective doses of antibiotics leading to further worsening. Our centre being a referral centre, the patients tends to reach us only after they have taken various course of antibiotics in due time and end up with multidrug resistant bugs.

Control of emergence of multidrug resistant bacteria is necessary as we have limited antibiotics, increasing immunocompromised population, increasing incidences of polymicrobial infections, the property of bacteria to transfer of resistant genes to other pathogenic bacteria and development of further resistance to penultimate group of antibiotics.

Furthermore, self medication, injudicious use of antibiotics, inadequate hospital infection control methods leads to increased resistance in bacteria. In

developing countries like ours, repeated, ineffective medication from local practitioners is a common practice especially in chronic diseases like chronic gall bladder diseases and this may be a cause of antibiotic resistance. These patients carrying community acquired strains, on admission to hospital exchange the genetic information with the prevailing nosocomial isolates, leading to multidrug resistant strains and polymicrobial infections.

CONCLUSION

Present study showed that preponderance of gram negative infections in the biliary tract continue to be a major threat in management of the patients with gall bladder disease. Also, the analysis of bile can be valuable in preparing a better treatment strategy in biliary tract infection. Rapid development of multidrug resistance in gram-negative bacteria causing biliary tract infections remains the cause of concern.

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