

Evaluation of Microbial Flora Present in the Bile of Patients with Biliary Diseases and Non-Biliary Diseases: A Comparative Study

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Abstract

Background: Gallstone disease is one of the most common disorders affecting the digestive system. Despite evidence suggesting that normal bile is sterile, bacteria are regularly found in inflamed gallbladders and in cholelithiasis patients. Therefore, choosing the appropriate drugs is aided by knowledge of and familiarity with the microorganisms found in gallbladder bile.

Methods: This prospective, single-center comparative study was conducted in Department of Surgery, in Maharshi Vashistha Autonomous State Medical college, Basti, for a period of 1 year from 15th March 2021 to 14th March 2022. In this study, total 100 patients were included and divided into study and the control group. The bile for culture was aspirated during the laparoscopic cholecystectomy and exploratory laparotomy. This bile was then cultured in the culture media and subsequently the sensitivity pattern was evaluated.

Result: The study results showed that females had more incidence of gall stone disease. In the study group the microbial positivity was 48%, compared to 6% in the control group. In 2 cases of the study group polymicrobial positivity was reported. The most frequent species, either by itself or in conjunction with Bacteroides, was E. coli. None of the gall bladder carcinoma patients obtained a positive culture result. Patients with cholecystitis, cholelithiasis, and choledocholithiasis showed the highest levels of positive.

Conclusion: This study showed that evaluation of presence bacterial species in the bile and subsequent the antibiotic management proved a key to achieve excellent results in patients with biliary diseases.

Keywords: Biliary diseases, gram-negative bacteria, microflora, antibiotic sensitivity

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Introduction

The prevalence of gall bladder disease varies greatly across different regions of the world. In the United States, for

example, an estimated 15 million people suffer with gall bladder stones, and of those people, one fifth undergo biliary

system surgery every day [1]. There are still many questions that need to be answered regarding the function that bacterial infections play in the various biliary disorders. One of the most common reasons people in India seek major abdominal surgery is because they have an illness of the biliary tract that is completely harmless. It has been recognized for a long time that bactibilia is connected with illnesses of the biliary tract, and culturable bacteria in bile can indicate a state of asymptomatic bactibilia, which can diffuse after any intervention and cause an infectious consequence [2].

In healthy people, the biliary tract is not likely to provide a breeding ground for bacteria for the most of the time. It is quite unlikely that there could be the same type of sparse indigenous flora that is present in the duodenum because full biliary blockage seldom coexists with bacterial cholangitis [3]. This would seem to be a very remote possibility. In trials with erythrocytes, it was demonstrated without a reasonable doubt that the primary effects of bile are exerted on the membranes of cells. Hemolysis is caused when bile is added, and as erythrocytes do not have organelles and do not have particular processes for the uptake and metabolism of bile salts, the lysis can only be attributed to membrane damaging effects because erythrocytes lack these organelles and mechanisms [4].

The presence of bacteria in the bile is associated with the development of gallstones in the gallbladder or biliary tree. Both the gallbladder and the biliary tree are capable of developing gallstones. Even in patients without stone disease, prior biliary surgery is linked to higher prevalence of bacteriobilia. When the biliary system is functioning normally, the presence of biliary system bacteria has no clinical significance [5]. Because of an obstruction in the bile duct, bacteria begin to multiply within the bile that is left to stand as the pressure in the biliary system

rises. It is possible that at some point in time the bacteria will translocate into the circulation, which will result in a systemic infection. This can happen at any time. Acute cholangitis has a continuous clinical spectrum and has the ability to progress from a local biliary disease to a progressive disease with sepsis and multiple organ dysfunction syndrome. This progression can occur at any time during the course of the disease. This evolution is possible at any point along the spectrum of experience [5].

In the pathogenesis of brown stones, bacterial infection has been considered as a primary factor. However, studies have also shown that even in formation of black stones bacterial infection acts as a primary factor. Most of the bacteria isolated from the bile is gram negative coliform bacteria, *E.coli* being the prime type [6]. Other types of coliform bacteria, such as *Klebsiella*, *Enterobacter*, and *Proteus*, are not isolated nearly as frequently. In addition to being regularly isolated, *Streptococcus faecalis*, often known as *enterococcus*, is an aerobic gram-positive coccus. In fewer than 10 percent of the cases, anaerobic bacteria are identified, with *Clostridium perfringens* being the most frequently found [6].

Anaerobic organisms cause the remaining six percent of bile tract infections. The vast majority of biliary tract infections are caused by aerobic species. Bacteria are frequently discovered in a gallbladder that is inflamed as well as in patients who suffer from cholelithiasis, despite the fact that data implies that normal bile is sterile. The permeability of an inflamed gallbladder is significantly altered, which enables the absorption of bile acids and the flow of inorganic ions into the gallbladder lumen [5]. It is possible that inflammation plays a function in the production of an excessive amount of cellular debris as well as an increase in the amount of protein that is secreted. In conclusion, bacterial enzymes have an effect on the constituents

of bile, which can change its solubility and ultimately cause the precipitation of bile salts. Most gall stones are composite in nature. Even the purest stones contain trace amounts of bacteria [7].

The purpose of this research was to examine the microbial flora of bile in patients who were suffering from biliary illnesses as opposed to those who had normal gall bladders. Additionally, the antibiotic sensitivity pattern of various types of bacteria that were taken from the bile of the patients who participated in this study was investigated and analyzed as part of this investigation.

Materials and methods

This prospective, single-center comparative study was conducted in Department of Surgery, in Maharashtra Vashista Autonomous State Medical College, Basti, for a period of 1 year from 15th March 2021 to 14th March 2022. One hundred individuals who were undergoing gastrointestinal surgery at some point during the course of this trial participated. From among these 100 individuals, 50 were considered to be in the study group since they had biliary or gall bladder illnesses. The remaining 50 patients had various gastrointestinal disorders that include normal gall bladder. These patients served as the study's control group because they did not have this condition.

Inclusion criteria

1. Age \geq 18 years.

Exclusion criteria

1. Patients with gall bladder empyema and gangrene
2. Systemic Inflammatory Response Syndrome (SIRS) or sepsis.
3. Patients who have used antibiotics in the past, including in the past three months.

Method

During the surgery, bile was aspirated and collected from the gall bladder for the purpose of culture using a sterile syringe with a 24-gauge needle. Full aseptic measures were observed throughout the process. During the exploratory laparotomy, bile from a normally functioning gall bladder was aspirated. There will be a collection of 6 milliliters of bile, and it will be transferred to the following containers:

- 2 ml of bile was transferred on 0.1% Thioglycollate or Robertson's cooked meat media. This was then sent for anaerobic culture.

- 2 ml of bile was transferred to Selenite F broth for aerobic culture where it will be incubated for 18-24 hrs at 37°C, remaining was directly transferred in a capped sterile container for aerobic culture. After that the incubated sample was examined for any growth of bacteria by its turbidity. Turbidity shows presence of bacterial growth. In case sample was not turbid then it was again incubated for 18-24 hrs at 37°C. After that it was subculture on following media:

- a. MacConkey's agar
- b. Blood agar
- c. Chocolate agar

After growth of the organisms, the colonies are further identified by biochemical tests and subsequently sensitivity pattern was studied.

- All the samples were incubated for 18-24 hrs at 37°C. After growth of organisms, the colonies were identified by biochemical tests and subsequently sensitivity pattern was studied.

Statistical analysis

The statistical analysis was done using SPSS (Statistical Package for Social Sciences) Version 15.0 statistical Analysis Software. The values were represented in Number (%) and Mean \pm SD. Appropriate

statistical analysis was done and then $P < 0.05$ were taken as significant.

Result

Total 100 patients were included in the study. A total of 50 (50%) subjects who had been operated upon for gastrointestinal disease apart from those affecting gall bladder and biliary tract comprised the control group of the study while the remaining 50 (50%) subjects who had been operated upon for biliary tract or gall bladder diseases comprised the study group.

It was shown that majority of subjects in both the groups were aged between 31 to 50 years.

However, in control group, the proportion of subjects in 51-60 years was significantly higher as compared to study group ($p=0.030$). In the study group majority of subjects were females compared to the control group where most of the participants were males. Gender wise, there was a significant difference between two groups ($p < 0.001$).

Table 1: Gender wise Distribution

S. No.	Gender	Control (n=50)		Study (n=50)	
		No.	%	No.	%
1.	Male	39	78	4	8
2.	Female	11	22	46	92

$$\chi^2=49.980 \text{ (df=1); } p < 0.001$$

In control group, there were only 28 (56%) patients with duration of present illness < 6 yrs as compared to 41 (82%) patients in study group. Statistically there was a significant difference between the two groups ($p=0.005$). The duration of present illness ranged from 5 to 12 yrs amongst control group subjects with a mean of 6.56 ± 1.25 years whereas amongst study

group subjects this duration ranged from 2 to 14 years with a mean of 5.60 ± 3.08 years.

All the cases in control group underwent exploratory laparotomy. In study group, 29 (58%) subjects underwent laparoscopic cholecystectomy, 15 (30%) underwent open cholecystectomy and 6 (12%) underwent open choledocholithotomy.

Table 2: Procedure

S. No.	Procedure	Number of subjects	Percentage
I. Control Group			
1.	Exploratory laparotomy	50	100
II. Study Group			
1.	Laparoscopic cholecystectomy	29	58
2.	Open cholecystectomy	15	30
3.	Open choledocholithotomy	6	12

When compared with the control group, the incidence of microbiological positive was discovered to be statistically substantially greater in the study group ($p < 0.001$).

Table 3: Microbial Positivity

S. No.	Positivity	Control (n=50)		Study (n=50)	
		No.	%	No.	%
1.	Positive	3	6	24	48
2.	Negative	47	94	26	52

$$\chi^2=22.374 \text{ (df=1); } p < 0.001$$

In the control group, out of the three samples that tested positive for microbes, 4% of those samples had *E. coli*, and 2% contained *Salmonella*. *E. coli* was the most prevalent type of microbia found in the bile of the study group's participants, whether it was found alone (n = 8; 16%) or in combination with *Bacteroides* sp. (n = 2; 4%).

Bacteroides sp. was the next most common microbia, either alone (n=4; 8%) or in

combination (n=2; 4%). Only 2 (4%) cases had co-infection of *E. coli* and *Bacteroides*. *Salmonella*, *Klebsiella*, *Enterobacter* and *P. aeruginosa* were the other microbia present in 2 (4%), 4 (8%), 3 (6%) and 1 (2%) sample. There were 47 (94%) specimen of Control group and 26 (52%) samples of study group who had no microbia present.

Table 4: Microbial Isolates

S. No.	Isolate	Control (n=50)		Study (n=50)	
		No.	%	No.	%
1.	<i>E. coli</i> alone	2	4	8	16
2.	<i>Salmonella</i>	1	2	2	4
3.	<i>E. coli</i> + <i>Bacteroides</i>	0	0	2	4
4.	<i>Bacteroides</i> alone	0	0	4	8
5.	<i>Enterobacter</i>	0	0	3	6
6.	<i>Klebsiella</i>	0	0	4	8
7.	<i>P. aeruginosa</i>	0	0	1	2
8.	None	47	94	26	52

The group that suffered from cholecystitis and cholelithiasis had the highest rate of positive (62.9%). Positive results for intestinal blockage and perforation peritonitis were found in 5.9% and 6.9% of cases, respectively. Subjects diagnosed with choledocholithiasis had a positive rate of 33.3%.

Table 5: Diagnosis and Microbial Flora

S. No.	Diagnosis	Total No. of patients	No. of patients with positive culture	Percentage
1.	Carcinoma gall bladder	9	0	0
2.	Cholecystitis with cholelithiasis	35	22	62.9
3.	Choledocholithiasis	6	2	33.3
4.	Intestinal obstruction	17	1	5.88
5.	Perforation peritonitis	29	2	6.9
6.	Volvulus	4	0	0

It was found that the incidence of positivity (43.5%) was significantly higher in patients of higher age group (>50 years) as compared to that (22.1%) in lower age group (<50 years).

Amongst various antibiotics assessed for sensitivity, Imipenem showed 100% sensitivity against all the microbial

species. Amikacin, Piperacillin+Tazobactam combination showed an overall sensitivity of 41.4%. Septran, Norflox and Nitrofurantoin did not show any sensitivity (0%). Tetracycline, Nalidixic acid, Furazolidone and Ceftazidime were found to be only 3.4% sensitive.

Table 6: Overall Sensitivity Pattern

S. No.	Antibiotic	% Sensitive	% Resistance
1.	Septran	0.0	100.0
2.	Tetracycline	3.4	96.6
3.	Chloramphenicol	20.7	79.3
4.	Ciprofloxacin	10.3	89.7
5.	Nalidixic acid	3.4	96.6
6.	Furazolidone	3.4	96.6
7.	Ampicillin	10.3	89.7
8.	Amoxicillin + Clavunic acid	20.7	79.3
9.	Gentamicin	24.1	75.9
10.	Amikacin	41.4	58.6
11.	Norflox	0.0	100.0
12.	Cefotaxime	13.8	86.2
13.	Cefepime	6.9	93.1
14.	Ceftazidime	3.4	96.6
15.	Cefoperazone + Sulbactam	10.3	89.7
16.	Nitrofurantoin	0.0	100.0
17.	Piperacillin + Tazobactam	41.4	58.6
18.	Imipenem	100.0	0.0
19.	Cefuroxime	6.9	93.1

In the present study, out of 50 cases in study group, 3 (6%) had more than >15 days of hospital stay. Two of these three had undergone open cholecystectomy while one had undergone open choledocholithotomy. None of the patients in whom laparoscopic cholecystectomy was done had hospital stay over 15 days. There was 1 expiry (2%). It was a patient of malignant carcinoma gall bladder. No other complication was reported in any of the patients in the study group. None of these patients had any infection.

Discussion

In present study, an assessment of microbial flora of subjects with normal gall bladder was made and compared with that of subjects undergoing treatment for gall-bladder disorders. Majority of patients in both study and control group were less than 50 years of age. Capoor et al.⁵⁴ have also reported the mean age of their patients to be 41 years [8].

In present study, the male to female ratio of the control group subjects was 3.55:1

whereas in study group this ratio was 0.09:1. In previous reports too, a higher prevalence of females has been reported for gall bladder disorders. Singh et al also reported a female predominance in their study [9]. In another study by Prabhu et al also female predominance were reported [5].

The difference in duration of illness between study and control group could be explained on the basis of the variation in the diagnosis of the two groups. In study group, carcinoma gall bladder patients usually adopt the surgical option after a long gap from the date of detection of illness.

In the current investigation, microbial positive in bile was found in 6% of control group patients who did not have any gall bladder related condition out of a total of 50 samples taken from each group. This proportion was significantly lower in the study group, where it was found in 24 patients out of 50 samples.

In a normal state, the bile does not have any gram-positive bacteria and the only microorganism present in the bile is the gram-negative bacteria [10]. In the current investigation as well, all three of the control group cases that showed microbial growth were discovered to include gram-negative bacteria. In one of these cases, Salmonella strains were recovered, while E. coli was detected in two of the three cases. The most recent instance included a patient who was diagnosed with typhoid fever, which ultimately led to perforated peritonitis.

In present study, none of the specimen in controls had a polymicrobial positivity, though there were two study groups in whom co-infection was observed. Both these cases had coinfection of E. coli and Bacteroides.

Al Harbi and colleagues collected bile samples from 112 patients who were undergoing elective laparoscopic cholecystectomy for gallstones. They discovered that 28 of these bile cultures (or 25 percent) were positive, and that four of these positive cultures contained more than one organism. On the other hand, the findings of the current investigation showed that microflora was present in 24 out of 50 (48%) of the bile samples [11].

According to the findings of Morris GJ and colleagues, the overall prevalence of live bacteria in bile samples taken during cholecystectomy was determined to be 15.6 percent [12]. In the study that Khan AB and colleagues conducted on 121 patients, they found that 34 percent of patients, or 42 out of 121, had a positive bile culture, whereas 66 percent, or 79 out of 121, had a negative bile culture [2].

Bactibilia was also discovered by Pushpalata H et al in 27 out of 50 (or 52 percent) of patients. It was discovered that 10% of the bile samples contained polymicrobial flora. The majority of the species that were found to be present were E. coli, Klebsiella pneumonia, and

Enterococcus faecalis [13]. One of the reasons for the relatively higher proportion of microbial positivity in our study could be the method of collection.

In patients with chronic cholecystitis but with no biliary blockage, Lotveit et al. investigated the function of juxta-papillary duodenal diverticula in gallbladder flora. Patients with diverticula had a considerably higher frequency of positive cultures than the control group. The samples that tested positive had bacteria from the gut flora. Because gallstones are so frequently found in patients with juxta-papillary duodenal diverticula, the authors hypothesized that bacteria may contribute to their development and that this ascending route of infection could lead to infection [14].

In control group, out of three samples with microbial positivity, 2 had E. coli and 1 (2%) had Salmonella. E. coli was the most common organism present in the bile either alone (n=8; 16%) or in combination with Bacteroides sp. (n=2; 4%) among patients in the study group. Bacteroides sp. was the next most common microbia, either alone (n=4; 8%) or in combination (n=2; 4%). Only 2 (4%) cases had co-infection of E. coli and Bacteroides. Salmonella, Klebsiella, Enterobacter and P. aeruginosa were the other microbia present in 2 (4%), 4 (8%), 3 (6%) and 1 (2%) sample. There were 47 (94%) specimen of Control group and 26 (52%) samples of study group who had no microbia present. No infection of any Gram-positive species was observed. Apart from Bacteroides all the bacteria were aerobes. Literature generally agrees on the presence of Gram-negative bacteria in bile. In the study of S family Propionibacteriaceae were more copious in bile samples from control subjects; on other way, in patients with cholelithiasis members of the families Bacteroidaceae, Prevotellaceae, Porphyromonadaceae, and Veillonellaceae were more commonly detected. [15].

Amongst various antibiotics assessed for sensitivity, Imipenem showed 100% sensitivity against all the microbial species. Amikacin, Piperacillin Tazobactam combination showed an overall sensitivity of 41.4%. Septran, Norflox and Nitrofurantoin did not show any sensitivity (0%). Tetracycline, Nalidixic acid, Furazolidone and Ceftazidime were found to be only 3.4% sensitive.

Capoor et al. in their study observed piperacillin-tazobactam and meropenem to be effective against majority of isolates including *E. coli*, *K. pneumoniae*, *C. freundii*, *P. aeruginosa* and *Acinetobacter* spp [8]. In present study we observed Imipenem to be 100% effective against all the isolates.

In present study, only three cases had hospital stay of >15 days, Two of these three had undergone open cholecystectomy while one had undergone open choledocholithotomy. None of the patients in whom laparoscopic cholecystectomy was done had hospital stay over 15 days. There was 1 expiry (2%). It was a patient of malignant carcinoma gall bladder. No other complication was reported in any of the patients in the study group. None of these patients had any infection. Thus, no association of type of infection with prolonged hospital stay or expiry was observed.

Conclusion

The findings in the present study suggest the presence of Gram-positive bacteria in general that too did not have a significant implication on the outcome. All the patients in the present study had a relatively shorter hospital stay and no major complication. Although results do not show a possible role of microbial flora as a prognostic indicator, however, unless otherwise effectively managed, these infections might have an impact on the well-being of patient. Thus, the results of

the present study are indicators and not conclusive. Further study with larger sample size is recommended to reach to a final conclusion in this direction.

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Authors Contribution

Dr Abhinav Chaudhary, Dr Abhishek Jina, Dr Lalmani Pal, and Dr Mahesh Chandra Pandey designed the research paper. Dr Abhinav Chaudhary, Dr Abhishek Jina analysed the data. Dr Abhinav Chaudhary wrote the paper and Dr Abhishek Jina, Dr Lalmani Pal, and Dr Mahesh Chandra gave substantial contribution. All the authors approved the final version.

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