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Microbiological Profile of Gall Bladder Bile in Cholecystectomy Patients

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Bile culture; Biliary microflora; Cholelithiasis; Cholecystectomy

1. Abstract

1.1. Introduction: The presence of gallstones in gallbladder or biliary tree is associated with the bacterial colonization of bile. Therefore, it is important to know the microbiological flora of bile before prophylactic antibiotics are given. The study was conducted to determine the bacteriology of aspirated bile samples taken during laparoscopic cholecystectomy, and to correlate it with the clinical profile of patients with symptomatic cholelithiasis.

1.2. Aim: To study the microbiological profile of gallbladder bile in cholelithiasis.

1.3. Patients and Methods: 100 patients undergoing laparoscopic cholecystectomy who met the inclusion criteria were included in the study, 10cc bile was extracted from the intact gall bladder using sterile syringe and needle. Sample was put in sterile culture bottle and transferred to laboratory for culture. Growth obtained was analysed for the causative agent identification.

1.4. Result: 31 patients showed positive bile culture in which Escherichia coli was the most common isolated bacteria and bile was sterile in 69 patients. Other organisms isolated were Pseudomonas, Klebsiella, Citrobacter, Acinitobacter and Proteus. Positive bile culture was a more common finding in patients with history of acute cholecystitis.

1.5. Conclusion: Culture of bile need to be necessary to prevent complication after cholecystectomy. In vast majority of patients, the bile was sterile and Escherichia coli was the most common isolated bacteria among positive bile culture group. The results of this study can help develop local guidelines and recommendations based on Indian data to ensure the rational use of prophylactic antibiotics in patients with symptomatic cholelithiasis.

2. Introduction

Biliary calculus disease is one of the most common disorder of gastrointestinal tract. The conditions that predispose to the development of gallstones are obesity, pregnancy, dietary factors, Crohn's disease, gastric surgery, hereditary spherocytosis, sickle cell disease, and thalassemia [1]. Most of the cases remain asymptomatic and only about 50% of patients are symptomatic within 5 to 20 years of diagnosis of gall stone disease [2]. Symptomatic patients present with complain on right upper abdomen pain, associated with nausea, vomiting and loss of appetite.

Gall stone disease has multifactorial pathogenesis, and it varies according to type of gallstones. Infective factor seems to be a major cause of formation of gallstones and the presence of gallstones within the gallbladder or biliary tree is associated with the bacterial colonization of the bile. Moynihan's aphorism that "gall stone is a tomb stone erected in the memory of the organism with in it" is true today [3].

Presence of gallstones within gallbladder or biliary tree is associated with bacterial colonization of the bile and bacteria can be cultured from gallbladder bile in about ½ cases of gallstone disease. These microorganisms reach gallbladder through blood stream, lymphatics or from infective focus elsewhere in the body [2]. Bacteria in the biliary system are of no clinical significance under conditions of normal bile flow. However, obstruction to bile flow leads to bacterial proliferation within the stagnant bile. Eventually, the bacteria presumably translocate into the circulation causing a systemic infection [4].

Microbiological infection has been considered a primary factor in the pathogenesis of brown and black pigment stones. Bacterial infection is the most common type of biliary tract infection, with a gram-negative preponderance. Gram-positive and anaerobic bac-

teria are uncommon causative agents. Viral and fungal agents are rare. The typical pathogens are the Gram-negative enteric aerobes such as Escherichia coli, Klebsiella species and Proteus species, while Pseudomonas aeruginosa, Bacteroides fragilis and Enterococcus faecalis are less commonly cultured. Bacteria are commonly found in inflamed gallbladder and in patients with cholelithiasis, whereas evidence suggests that normal bile is sterile.

Preoperative and postoperative antibiotics are commonly used in patients undergoing Cholecystectomy, without demonstrating specific bacterial predominance, its resistance or sensitivity. Microbial resistance is a growing public health problem associated with increased morbidity and mortality for both patients and institutions. The inappropriate use of antibiotics is the principal cause of microbial resistance. Therefore, the frequency of biliary infection in patients undergoing Cholecystectomy needs to be established.

This study was designed to evaluate the incidence of biliary microflora in gall stone disease and most common biliary microflora in patients undergoing laparoscopic cholecystectomy for gall stone disease. The resultant data will prove beneficial in identifying the causative agents of Bile infection in our hospital setting. It will be helpful in accurate antibiotic therapy, infection control protocols and prevent prolonged hospital stay.

3. Patients and Methods

3.1. Study Design

A Hospital based case series analysis, conducted between 1st May 2020 to 30th April 2021 in Department of General Surgery in Shri Mahant Indiresh Hospital and Shri Guru Ram Rai Institute of Medical & Health Sciences, Dehradun, Uttarakhand.

3.2. Inclusion Criteria

1. Patients of all age and both sexes.

2. Patients presenting with Cholelithiasis undergoing laparoscopic cholecystectomy.

3.3. Exclusion Criteria

1. Patients with Cholelithiasis with other complications-

- Choledocholithiasis
- Acute Cholecystitis
- Cholangitis
- Empyema Gall Bladder
- Mucocele Gall Bladder

2. Patient who has Intra Operative biliary spillage.

3.4. Methodology

- The general demographic details of patient regarding their name, age, sex, occupation, socio-economic status, and address were collected.
- A detailed history was taken with special reference to abdominal pain and its associated symptoms.
- A relevant general physical examination, abdominal and systemic examination was done.
- Ultrasonogram was performed on all patients to confirm the clinical diagnosis of Cholelithiasis. A routine pre-anaesthetic check-up was done.
- A fully explained well informed written consent was taken.

Sample collection:

- After separating gall bladder from liver bed, intact gall bladder was extracted from epigastric port partially using gall bladder extractor.
- Gall bladder was clamped using artery forceps and 10ml bile was extracted from the intact gall bladder using sterile syringe and needle.
- The sample was put in a sterile culture bottle (yellow top. Aerobic Blood culture bottle, BacT Alert, bioMerieux) and was transferred to laboratory for culture.
- Sample from Culture positive bottles was further processed on Blood agar and Mac Conkey agar and incubated for 48hrs at 37.C.
- Growth thus obtained was analysed for the causative agent using automated method for identification (Vitek-2 compact, bioMerieux).
- Result thus obtained was compiled and analysed (Figure 1).

Figure 1: VITEK 2 Compact

4. Observation and Result

A total of 100 patients admitted in Department of General Surgery in Shri Mahant Indiresh Hospital and Shri Guru Ram Rai Institute of Medical & Health Sciences, Dehradun, Uttarakhand, who met the inclusion criteria and underwent laparoscopic cholecystectomy were included in the study. An evaluation was undertaken to study Bacteriological analysis of bile.

Out of 100 patients, 54 (54.0%) patients belonged to age group of 18-40 years, 35 (35.0%) patients belonged to age group of 41-60 years and 11 (11.0%) patients belonged to age group of above 60 years (Table 1).

Out of 100 patients, there were 20 (20.0%) males and 80 (80.0%) females. The male to female ratio was 1:4 (Figure 2).

Pain in upper abdomen was the most common symptom, present in all patients (100%). Site of pain abdomen was observed as right hypochondrium in 68 (68.0%) patients and epigastric region in 32 (32.0%) patients, most common associated symptom was anorexia seen in 65 (65.0%) patients, followed by nausea and vomiting seen in 59 (59.0%) patients.

Out of 100 study participants, Culture reports of the bile revealed organism in 31 cases (31%) while it showed no growth of organism in 69 cases (69%), demonstrating that bile is sterile in majority of cases (Table 2 and Figure 3).

Among the culture positive cases (31). E. Coli was the most common organism isolated from 12 patients (38.7%) followed by Pseudomonas putida 6 (19.4%), Pseudomonas aeruginosa 4 (12.9%), Citrobacter freundii 3 (9.7%), Acinetobacter baumanii 2 (6.5%), Klebsiella pneumoniae 2 (6.5%), Proteus mirabilis 2 (6.5%) (Figure 4).

20 cases had history of Acute cholecystitis, out of which bile culture was positive in 14 cases and no growth was seen in 6 cases, which was statistically significant, showing that patients with past history of Acute cholecystitis had increased chances of bile culture growth positivity.

Table 1: Distribution of study participants undergoing according to age.

AGE GROUPS	FREQUENCY (N=100)	PERCENTAGE
18-40 years	54	54%
41-60 years	35	35%
> 60 years	11	11%
TOTAL	100	100%

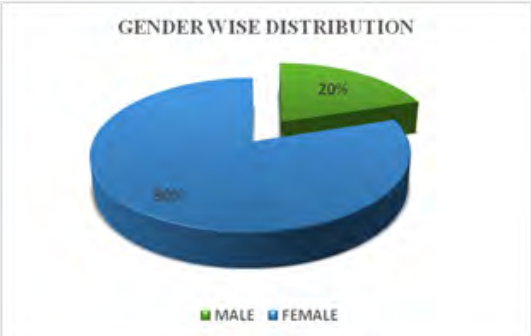

Figure 2: Gender wise distribution of study participants.

Table 2: Bacteriology of Bile Culture in Gall Stone Disease.

BILE CULTURE ORGANISM	FREQUENCY (N = 100)	PERCENTAGE
No growth	69	69.00%
Growth Present	31	31.00%

Figure 3: Growth on Mac Conkey Agar.

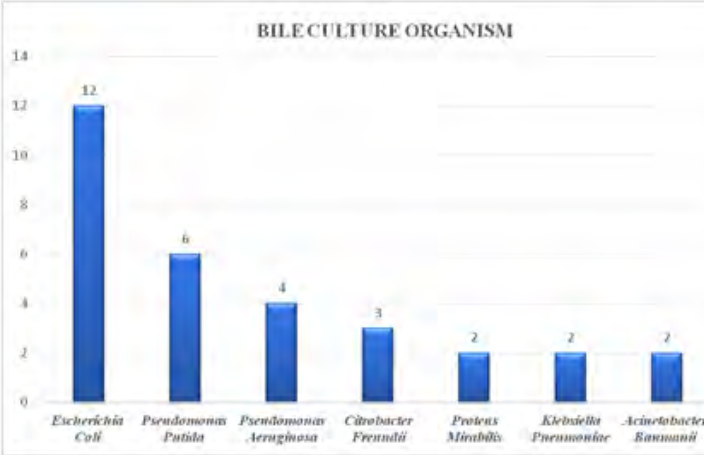

Figure 4: Frequency of Bile Culture organism in Gall Stone Disease.

5. Discussion

In our study the mean age of the study population was 41.7±12.63 (22.00-80.00) years which was in conjunction with the work done by Arora et al [5] and Shukla A et al [6]. Out of the 100 study participants 20.0% (20) were males and 80.0% (80) females with male to female ratio of 1:4. Similar results were seen in the study conducted on microbiology of bile by Ahmad et al [7], in which the age distribution ranged from 16 to 80 years (mean age: 46.26 years) and 28 patients were males (26.4%) and 78 (73.6%) were females with male to female ratio of 1:2.8. Similar observations were found by Sharma et al [8] who conducted study on bacteriological analysis of bile and included 102 patients in their study, 75(73.52%) were female and 27(26.47%) were male, with M:F ratio of 1:2.8.

As per literature gall stone disease is more common in fat, fertile, female of forty and similar findings were seen in the present study.

Most patients remain asymptomatic from their gallstones. However once symptomatic, patients tend to have recurring symptoms, usually repeated episodes of biliary colic that may be associated with nausea and vomiting. The pain is most commonly triggered by fatty foods, but it can also be initiated by other types of food or even occur spontaneously.

Patients in the present study presented with varied signs and symptoms and most of the patients were having multiple symptoms at the time of presentation, the most common clinical presentation was abdominal pain, present in all the cases studied (100%). The second most common presentation was anorexia, seen in 65 (65%) cases, followed by nausea/vomiting seen in 59 (59%) cases. These results were similar to the study of Ramos et al [9] on incidence of bacteria from cultures of bile, out of the 183 patients studied, 100% presented with initial symptoms of colic-type pain in the right hypochondrium and epigastrium and 74 patients had associated nausea and vomiting (40.4%).

In a study conducted by Suri et al [10] on biliary bacteriology, the patients presented with varied signs and symptoms, most of the patients had an overlap of presenting symptoms.; abdominal pain was the commonest symptom (97.3%), followed by fatty-food intolerance (61.3%), nausea (30%), vomiting (23.3%) and dyspepsia (20.6%).

In the present study, 100 bile samples were analysed for the presence of microorganisms out of which 31 (31%) showed bacterial growth and 69 (69%) cultures were sterile. This was in accordance with studies conducted by Hadi YB et al [11] (bactibilia in acute cholecystitis) and Capoor MR et al [4] (Microflora of bile aspirates) who found bacterial growth rate of (33.6%) and (32%) cases respectively.

Gram-negative aerobes are the organisms most frequently isolated from bile in patients with symptomatic gallstone disease. In our study, E. coli was the most commonly isolated microorganism, identified in 12 out of 31 patients (38.7%). Other microorganisms isolated were Pseudomonas putida in 6 out of 31 cases (19.4%), Pseudomonas aeruginosa in 4 out of 31 cases (12.9%), Citrobacter freundii in 3 out of 31 cases (9.7%), Acinetobacter baumanii in 2 out of 31 cases (6.5%), Klebsiella pneumoniae in 2 out of 31 cases (6.5%) and Proteus mirabilis in 2 out of 31 cases (6.5%).

Similar results were also seen in other studies, in a study conducted by Ahmad et al [7] on microbiology of bile, most commonly cultured organism was E coli, cultured in 10 (40%) patients, followed by Klebsiella in 5 (20%) patients, Pseudomonas in 5 (20%) patients, Proteus and Staphylococcus aureus in 2 (8%) patients and mixed organisms (E coli and Pseudomonas) were cultured in 1 (4%) patient. In another study conducted by Kaur et al [12] on microbiological profile of biliary tract infections- the organisms obtained were Escherichia coli (40%), Klebsiella spp. (20%), Pseudomonas spp. (16%), and coagulase-negative-Staphylococci (8%).

In study conducted by Parekh et al [13] on bacteriological analysis of bile- Gram-negative aerobes were the organisms most frequently isolated from bile in patients with symptomatic gallstones. E. coli (15.38%) was the most common organism isolated from bile cultures. Other organisms were Pseudomonas (3.85%), Klebsiella (2.56%), coagulase negative Staphylococcus (1.28%) and Staphylococcus viridans (1.28%). Similar results were seen in a study conducted by Sharma et al [8] on bacteriological analysis of bile culture. In their study, Escherichia coli (37.03%) was the most common organism isolated from bile cultures. Other organisms were Pseudomonas sp. (29.62%), Klebsiella sp. (14.81%) and Citrobacter sp. (11.11%).

In a study by Capoor et al [4] on microflora of bile aspirates, the most common organisms isolated were Escherichia coli (11, 29.7%), Klebsiella pneumoniae (10, 27%), Citrobacter freundii (3, 8.1%), Salmonella enterica serovar typhi (3, 8.1%), Pseudomonas aeruginosa (2, 5.4%), Acinetobacter spp. (1, 2.7%), Candida krusei (1, 2.7%), Staphylococcus aureus (1, 2.7%). Polymicrobial infection of P. aeruginosa with K. pneumoniae was observed in 4 patients (3.8%).

Normally bile is sterile because of various anatomical and physiological mechanisms. A competent sphincter of Oddi prevents intestinal contents from refluxing into the bile duct, and anterograde bile flow periodically flushes the biliary system, keeping it free of organisms. In addition, bile components including bile salts and immunoglobulin A (IgA) have antibacterial characteristics protecting the biliary tree from bacteria. Prolonged bile duct obstruction leads to impaired intestinal wall barrier function. Consequently, bacteria can colonize bile easier.

So, from this compiled data we can conclude that in majority of cases bile is sterile, and in infected bile the enteric Gram-negative aerobes are the commonly found organisms. E. coli was the most common organism isolated in our study and in other studies conducted on bile culture. However other microorganisms seen in bile culture growth are variable in occurrence, although majority of them are enteric Gram-negative aerobes.

In our study statistically significant correlation was found between bile culture positivity and past history of acute cholecystitis. Out of 100 study participants 20 cases had history of Acute cholecystitis, out of which bile culture was positive in 14 cases and no growth was seen in 6 cases, which was statistically significant, with highly significant p value <0.001. Similar results were in study conducted by Suri et al [10] to study biliary bacteriology, in their study highest incidence of positive cultures was noted in patients with acute cholecystitis (40%). Sharma et al [8] in a study on bacteriological analysis of bile culture found that out of the 27 culture positive cases, 8(29.62%) were of acute cholecystitis and 19(70.37%) were of chronic cholecystitis. These observations points toward infection as a cause of bactibilia.

6. Conclusion

Gallstone disease is one of the most common problem affecting the digestive tract. Laparoscopic cholecystectomy is now considered the treatment of choice for symptomatic gallstone disease. This study was done to study the microbiological profile of gall bladder bile in patients undergoing laparoscopic cholecystectomy. 100 study participants who met the inclusion criteria were included in this.

Our study demonstrated that bile in majority of cases was sterile (69%) and growth of microorganism was seen in 31% study participants. E coli (38.7%) was the most common microorganism isolated from bile culture, detected in 12 out of 31 study participants with bile culture growth, other microorganisms isolated were Pseudomonas putida (19.4%), Pseudomonas aeruginosa (12.9%), Citrobacter freundii (9.7%), Acinetobacter baumanii (6.5%), Klebsiella pneumoniae (6.5%) and Proteus mirabilis (6.5%) cases among bile culture positive group.

In our study positive bile culture was a more common finding in patients with history of acute cholecystitis (14 out of 20). Which was also statistically significant with P value of <0.001.

From the above results, it is concluded that gall stone disease affect females far more than males and bile infection often complicates gall stone diseases and overall gram-negative organisms predominated in gall bladder bile culture and it was more common in patients with history of acute cholecystitis. Apart from surgery prompt administration of appropriate antibiotics to control the biliary tract infection is also very important and culture of bacteria from bile need to be necessary to prevent complication after cholecystectomy.

The results of this study can help develop local guidelines and recommendations based on Indian data to ensure the rational use of prophylactic antibiotics in patients with symptomatic cholelithiasis and this will help in preventing morbidity and mortality among patients undergoing laparoscopic cholecystectomy.

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