

## BACTERIAL FLORA OF BILE FROM GALL BLADDER OF PATIENTS UNDERGOING ELECTIVE LAPAROSCOPIC CHOLECYSTECTOMY FOR GALLSTONE DISEASE

Sushant Trakroo<sup>1</sup>, Sreejan Sharma<sup>2</sup>, Zahur Hussain<sup>3</sup>, Shavi Rayoo<sup>4</sup>, Shahnawaz Ahmed Chowdhary<sup>5</sup>

Received : 01/06/2023  
Received in revised form : 11/07/2023  
Accepted : 25/07/2023

**Keywords:**

Bile culture, gallstone disease, cholecystectomy, antibiotic sensitivity.

**Corresponding Author:**

Dr. Shahnawaz Ahmed Chowdhary,  
Email: trakroo.sushant@gmail.com.

DOI: 10.47009/jamp.2023.5.4.187

Source of Support: Nil,  
Conflict of Interest: None declared

*Int J Acad Med Pharm*  
2023; 5 (4); 925-928



<sup>1</sup>Senior Resident, Department of General Surgery, Government Medical College Jammu, India.

<sup>2</sup>Senior Resident, Department of General Surgery, Government Medical College Jammu, India.

<sup>3</sup>Professor, Department of General Surgery, Government Medical College Jammu, India.

<sup>4</sup>Junior Resident, Department of General Surgery, Government Medical College Jammu, India.

<sup>5</sup>Senior Resident, Department of General Surgery, Government Medical College Jammu, India.

### Abstract

**Background:** Gallstone disease poses a serious public health burden. There are a variety of probable pathways for bacterial migration and colonization of the biliary tract, such as translocation through the Oddi sphincter from the duodenum or hematogenous entrance into the liver with subsequent excretion into the bile. The presence of bacteria in the gallstones and in the bile of patients suffering from gallstone disease can lead to various complications hence making the study of bacterial flora from bile vitally important. **Materials and Methods:** A prospective time study was conducted over a period of one (01) year from 1st November 2020 to 31 October 2021. Bile was aspirated from the excised gall bladder and was sent in the sterile container in the microbiological laboratory for culture. The socio-demographic and clinical profile of the study participant was recorded for the purpose of analysis. Further both quantitative and qualitative data were retrieved meeting both objectives and the same was subjected to statistical analysis. **Results:** The age group of 31 – 40 years and females were more commonly affected. The most common pre-operative event was acute cholecystitis i.e. 06 out of 49 (12.24%), followed by acute pancreatitis in 04 patients (8.16%). The findings suggest that culture reports of the bile revealed organisms in 14 cases (28.57%) while it showed no growth of organisms in 32 cases (65.30%) whereas in 03 cases the sample was contaminated. E.Coli was the most common organism in 10 patients (20.40%) followed by Klebsiella in 6 patients (12.24 %), Proteus vulgaris in 3 patients (6.12%), Enterococcus in 03 patients (6.12%), Streptococcus in 1 patient (2.04 %) and Salmonella in 01 patient (2.04%). In the culture and sensitivity test, E.Coli showed maximum sensitivity to Amikacin in 7 cases (26%) followed by Piperacillin in 6 patients (22.2%). Klebsiella showed high sensitivity to Meropenem in 4 (21%) patients, Proteus vulgaris showed the highest sensitivity to Meropenem in 3 cases (33%) followed by Piperacillin and Amikacin (22.22%) cases. Salmonella was found in the bile culture of 1 patient. The case was highly sensitive to ceftriaxone, ciprofloxacin, and azithromycin. Enterococcus showed the highest sensitivity to Cefotaxime in 2 (25%) patients. Streptococcus showed equal sensitivity to Meropenem, Ciprofloxacin, Amikacin, and Cefotaxime i.e. 25 % in all the cases. Pseudomonas showed high sensitivity to Meropenem in 3 (83%) patients followed by Amikacin and Piperacillin (20%). **Conclusion:** The current study revealed a substantially high incidence (28.75) of bacteria in the bile of the patients with Gall Stone Disease. E. coli was the most common organism followed by Klebsiella, Proteus vulgaris, Enterococcus, Streptococcus, and Salmonella. E.coli showed maximum sensitivity to Amikacin followed by Piperacillin. Klebsiella showed high sensitivity to Meropenem, Proteus vulgaris showed the highest sensitivity to Meropenem followed by Piperacillin and Amikacin. Salmonella was highly sensitive to ceftriaxone, ciprofloxacin, and azithromycin. Enterococcus showed the highest sensitivity to Cefotaxime. Streptococcus showed equal sensitivity to Meropenem, Ciprofloxacin, Amikacin, and Cefotaxime. Pseudomonas showed high sensitivity to Meropenem followed by Amikacin and Piperacillin.

## INTRODUCTION

Gallstones are hardened deposits of digestive fluid inside the gallbladder. Gallstones develop in various sizes, shapes, and numbers. Generally, patients with gallstones don't exhibit the symptoms.<sup>[1]</sup>

Gallstone disease is a very common medical condition worldwide, affecting the gastrointestinal tract. Gallstone is the most common cause of hospital admission and to be treated surgically. Gallstones develop inside the gallbladder or biliary tract and obstruct the bile pathway. Most of the patients are asymptomatic in the initial stages and the patients may be symptomatic or asymptomatic; when the patient is having the symptoms or complications defined as gallstone disease. Symptomatic patients with gallbladder stones present episodes of right-upper-quadrant or epigastric pain which is accompanied by nausea and vomiting which aggravate for 30 minutes to hours and this is the result of the impaction of the stone.<sup>[2]</sup>

Gallstones can be either cholesterol or pigment (or bilirubinate)-based. Changes in hepatic and gallbladder function lead to the formation of cholesterol stones in the gallbladder. The primary metabolic precursor of cholesterol gallstones is excessive liver production of cholesterol, which can be brought on by medicines, obesity, or other reasons. Hypomotility and the release of nucleating agents such as mucus glycoprotein are two aspects of the gallbladder that contribute to stone formation.<sup>[3]</sup>

Some predisposing factors contribute in developing gallstones, i.e. sedentary lifestyle, gender, pregnancy, dietary factors, Crohn's disease, gastric surgery, hereditary, etc. As there is the development of stones in the biliary tract, the inflamed gallbladder allows the bacteria to grow more.<sup>[4]</sup>

Bile is a fluid that is released by the liver and stored in the gallbladder and helps in digestion, it's sterile in normal healthy conditions. There is a <30% chance of isolating bacteria in acute cholecystitis.<sup>[5]</sup>

As there is inflammation of the gallbladder, septic complications are acknowledged from stone development. The infective process originating from gallstones is independent of contamination of the peritoneal cavity with infected bile. As well as the chemical composition of gallbladder stones also has a significant influence on complications and is common when brown pigment stones are retained in the abdominal cavity.<sup>[6]</sup>

Rapidly emerging resistant bacteria threaten the extraordinary health benefits that have been achieved with antibiotics.<sup>[7]</sup>

Mixed gallstones are frequently associated with cholecystitis. Bile which is normally sterile is found to be positive in 50% of those with gallstone disease. These bacteria reach the gallbladder via the bloodstream from infective focus found elsewhere in the body. The biliary infection can be caused by any type ranging from aerobic gram-positive to gram-negative to anaerobic organisms. Aerobic causes

94% of biliary tract infections while anaerobic causes the rest. The most common organism includes *E. coli*, *Klebsiella*, and *Streptococcus*.<sup>[8]</sup>

Different reasons for biliary tract infection have been presented, e.g. ascending infection due to reflux of duodenal contents, blood-borne infection, and infection spread through the portal-venous channels. Ascending infection from the duodenum is thought to be the primary mechanism by which bacteria enter the bile.<sup>[9]</sup>

The gallbladder stones have a low mortality rate but a high morbidity rate in developing countries. Due to obstruction, bile becomes infected. In a research study, it was found that among 100 patients who underwent cholecystectomy 36% of patients had bactobilia. Further, it is reported that the prevalence of bactobilia in 20% of patients with microorganisms such as *Escherichia coli* (*E.coli*) (40%), *Klebsiella* (35%), *Salmonella* (20%) and *Shigella* (20%) who underwent cholecystectomy.<sup>[10]</sup>

Bactobilia has long been known to be associated with biliary tract diseases and culturable bacteria in bile can represent a state of asymptomatic bactobilia which can disseminate after any intervention causing infective complications. Exploring the microflora of the gall bladder, bile plays an important role in choosing the appropriate antibiotic to prevent complications.

## MATERIALS AND METHODS

A prospective time study was conducted over a period of one (01) year after Indian Ethical Committee permission (vide no. ECR/454/Inst/JK/2013/RR-20, Dated 26-05-2020). A written well-informed consent was taken and the nature & purpose of the study was explained. All bioethics/clinical research principles laid down under good clinical practice were followed.

Study Design: Prospective Study

Duration of Study: One year (1st November 2020 to 31 October 2021).

### Inclusion Criteria

All patients between the age group of 18 years to 70 years were admitted for elective laparoscopic cholecystectomy in GMC, Jammu in the given period.

### Exclusion Criteria:

1. Patients less than 18 years and more than 70 years.
2. Pregnant females.
3. Coagulation abnormalities.

Bile was aspirated from the excised gall bladder and was sent in the sterile container in the microbiological laboratory for culture. The socio-demographic and clinical profile of study participant was recorded for analysis. Further both quantitative and qualitative data were retrieved meeting both objectives and the same was subjected to statistical analysis.

## RESULTS

- In the present study, the age group of 31 – 40 years (16 cases) was more commonly affected (32.6%), followed by 41-50 (30%) and 21-30 years (18%) age group, and females were more commonly affected. Out of 49 patients, 35 (71.4%) were females and males were 14 cases (28.57%).
- Among 49 cases reported, 16 cases reported comorbidities. The most common comorbidities among the patients were Hypertension and Diabetes Mellitus i.e. 4% of the patients have Hypertension and Diabetes Mellitus respectively. Out of 49 patients, 33 patients i.e. 67.34 % have not reported any type of comorbidity.
- In 49 patients, 43 patients (88%) have reported delineated calot's triangle whereas 06 patients (12%) have frozen clot triangle. The most common pre-operative event was acute cholecystitis i.e. 06 out of 49 (12.24%), followed by acute pancreatitis in 04 patients (8.16%). Typhoid fever was also been reported among 01 patients i.e. 2.04% of the total cases studied. Further, 38 (77.5%) patients had no reported pre-operative event.
- The findings suggest that on culture reports of the bile revealed organisms in 14 cases (28.57%) while it showed no growth of the organism in 32 cases (65.30%) whereas in 03 cases the sample was contaminated. E.coli was the most common organism in 10 patients (20.40%) followed by Klebsiella in 6 patients (12.24 %), Proteus vulgaris in 3 patients (6.12%), Enterococcus in 03 patients (6.12%), Streptococcus in 1 patient (2.04 %) and Salmonella in 01 patient (2.04%).
- The culture and sensitivity test, E.coli showed maximum sensitivity to Amikacin in 7 cases (26%) followed by Piperacillin in 6 patients (22.2%). Klebsiella showed high sensitivity to Meropenem in 4 (21%) patients, Proteus vulgaris showed the highest sensitivity to Meropenem in 3 cases (33%) followed by Piperacillin and Amikacin (22.22%) cases. Salmonella was found in the bile culture of 1 patient. The case was highly sensitive to ceftriaxone, ciprofloxacin, and azithromycin. Enterococcus showed the highest sensitivity to Cefotaxime in 2 (25%) patients. Streptococcus showed equal sensitivity to Meropenem, Ciprofloxacin, Amikacin, and Cefotaxime i.e. 25 % in all the cases. Pseudomonas showed high sensitivity to Meropenem in 3 (83%) patients followed by Amikacin and Piperacillin (20%).

## DISCUSSION

Gallstones are hardened deposits of concentrated bile inside the gallbladder. Gallstones develop in various sizes, shapes, and numbers. Generally, patients with gallstones don't exhibit the symptoms.

In the present study, the age group of 31 – 40 years (16 cases) were more commonly affected (32.6%), followed by 41-50 (30%) and 21-30 years (18%) age group. Out of 49 patients, 35 (71.4%) were females and males were 14 cases (28.57%).

In a similar study conducted, the mean age of 55.6+14.3 years (range; 18 and 63 yr), and study population included 88 (66.7%) females and 44 (33.3%) males.<sup>[11]</sup>

In the present study culture reports of the bile revealed organisms in 14 cases (28.57%) while it showed no growth of organisms in 32 cases (65.30%) whereas in 03 cases the sample was contaminated.

E. Coli was the most common organism in 10 patients (20.40%) followed by Klebsiella in 6 patients (12.24 %), Proteus vulgaris in 3 patients (6.12%), Enterococcus in 03 patients (6.12%), Streptococcus in 1 patient (2.04 %) and Salmonella in 01 patient (2.04%).

In a similar studies conducted, authors found that the most common organism cultured was Escherichia coli followed by Enterococcus followed by Klebsiella pneumonia, and Pseudomonas species.<sup>[12,13,14,15]</sup>

In another study conducted, among the total 132 bile samples, bacteria were isolated in 50 samples (37.87%). Anaerobic bacteria were detected in 8 (16%) patients; Bacteroides fragilis (2; 4%) and Bacteroides perfringens (6; 12%). Escherichia coli was the most common isolate (13; 26%). Enterobacter was isolated from 9 samples (18%) followed by (13; 26%). Enterobacter was isolated from 9 samples (18%) followed by Salmonella typhi (7; 14%), Coagulase-negative Staphylococcus (6; 12%), Klebsiella pneumonia (2; 4%), Proteus (2; 4%). Salmonella typhi was isolated only from samples of female patients.<sup>[11]</sup>

It was found on culture and sensitivity test, E.coli showed maximum sensitivity to Amikacin in 7 cases (26%) followed by Piperacillin in 6 patients (22.2%). Klebsiella showed high sensitivity to Meropenem in 4 (21%) patients, Proteus vulgaris showed the highest sensitivity to Meropenem in 3 cases (33%) followed by Piperacillin and Amikacin (22.22%) cases. Salmonella was found in the bile culture of 1 patient. The case was highly sensitive to ceftriaxone, ciprofloxacin, and azithromycin. Enterococcus showed the highest sensitivity to Cefotaxime in 2 (25%) patients. Streptococcus showed equal sensitivity to Meropenem, Ciprofloxacin, Amikacin, and Cefotaxime i.e. 25 % in all the cases. Pseudomonas showed high sensitivity to Meropenem in 3 (83%) patients followed by Amikacin and Piperacillin (20%).

In a similar study conducted by, most of the organisms show sensitivity against cefoperazone/sulbactam or piperacillin/ tazobactam combination.<sup>[18]</sup>

In another study conducted by, among them, E. coli was positive in 6, and Klebsiella in 4. And the organisms were sensitive to Colistin (26.3%) and

Imipenem (23.7%) accordingly. And a resistance for Amikacin and Gentamycin was noted.<sup>[19]</sup>

## CONCLUSION

The current study revealed a substantially high incidence (28.75) of bacteria in the bile of the patients with Gall Stone Disease. E.coli was the most common organism followed by Klebsiella, Proteus vulgaris, Enterococcus, Streptococcus, and Salmonella. E.coli showed maximum sensitivity to Amikacin followed by Piperacillin. Klebsiella showed high sensitivity to Meropenem, Proteus vulgaris showed the highest sensitivity to Meropenem followed by Piperacillin and Amikacin. Salmonella was highly sensitive to ceftriaxone, ciprofloxacin, and azithromycin. Enterococcus showed the highest sensitivity to Cefotaxime. Streptococcus showed equal sensitivity to Meropenem, Ciprofloxacin, Amikacin, and Cefotaxime. Pseudomonas showed high sensitivity to Meropenem followed by Amikacin and Piperacillin.

## REFERENCES

1. Channa N A, Khand F D, Khand T U et al. Analysis of human gallstone by fourier transform infrared (FTIR). Pak J Med Sci 2007; 23:546–50.
2. Lammert F., Gurusamy K., Ko C., et al., Gallstones. Nat Rev Dis Primers 2016; 2, 16024.
3. Bouchier IA. The formation of gallstones. Keio J Med. 1992 Mar;41(1):1-5. doi: 10.2302/kjm.41.1. PMID: 1583812.
4. Prabhu T, Chandan CS, Sudarsan S. Microflora of gall bladder bile in patients undergoing laparoscopic cholecystectomy. International Surgery J 2018; 5(8):2876
5. Yun Pil Sung , Seo Hyung-II. Clinical aspects of bile culture in patients undergoing laparoscopic cholecystectomy. Medicine 2018; 97(26):e11234.
6. Hazrah P., KTH Oahn, Tewari M, et al. The frequency of live bacteria in gallstones. HPB 2004; 6(1): 28-32.
7. Bartlett JG, Gilbert DN, Spellberg B. Seven ways to preserve the miracle of antibiotics. Clin Infect Dis. 2013;56(10):1445–1450.
8. Gupta AM, Ramteke S, Kanwar KS, Soni P. Study of morphological spectrum of gallstone and bacteriology of bile in cholelithiasis . Int Surg J 2017;4:177-80.
9. FH Fukunaga. Gallbladder bacteriology, histology, and gallstones. Study of unselected cholecystectomy specimens in Honolulu. Arch Surg 1973;106 (2):169-71.
10. PRL Gomes, SSN Fernando, DD Weerasekara, et al. Aerobic bacteria associated with symptomatic gallstone disease and their antimicrobial susceptibility. Galle Med J 2006;11(1):9-13.
11. Bistgani Moazeni Mohammad, Imani Reza. Bile Bacteria of Patients with Cholelithiasis and Theirs Antibiogram. Acta Medica Iranica 2013; 51(11): 779-783.
12. Pitt HA, Postier RG, Cameron JL. Biliary Bacteria: Significance and Alterations After Antibiotic Therapy. Arch Surg. 1982;117(4):445–449. doi:10.1001/archsurg.1982.01380280037008.
13. R Malini, Capoor Deepthi, Nair Rajni, et al. Microflora of bile aspirates in patients with acute cholecystitis With or without cholelithiasis: a tropical experience. Braz J Infect Dis 2018; 12 (3):222-5.
14. Ohdan H, Oshiro H, Yamamoto Y, Tanaka I, Inagaki K, Sumimoto K, Hinoi T. Bacteriological investigation of bile in patients with cholelithiasis. Surg Today. 1993;23(5):390-5. doi: 10.1007/BF00309495. PMID: 8324331.
15. M Harbi AL, O A Osoba, A Mowallad, et al. Tract microflora in Saudi patients with Cholelithiasis. Trop Med Int Health 2001; 6(7):570-74.
16. Hussain P A Shinas, Kumar Santhosh, R M Rajesh Microbiological Flora Assessment of Bile in Elective Cholecystectomy. Kerala Surg J 2018.
17. Shetty Sudhakar Abhijith, Prabhakar Sonali, S M Moosabba. Bile culture and sensitivity in post cholecystectomy specimens. Med Plus- Research Publication. 2017; Vol. 3(1):16-18