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**Research Article**

# Bacteriological analysis of bile in cholecystectomy patients

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## ABSTRACT

**Background:** Cholecystectomy is currently a frequently performed operation. The presence of gallstones within either the gallbladder or biliary tree is associated with the bacterial colonization of the bile. Acute cholangitis spans a continuous clinical spectrum and can progress from a local biliary infection to advanced disease with sepsis and multiple organ dysfunction syndrome. Therefore, it is important to know the microbiological flora of the gallbladder before prophylactic antibiotics are given. Aims & objectives: To evaluate the microbiological profile of bile from gall bladder in patients undergoing cholecystectomy. To determine the appropriate antibiotic for preoperative prophylaxis in cholecystectomy patients based on the microbiological profile of bile.

**Methods:** The study was a prospective study carried out in SSG Hospital. A total of 78 patients undergone cholecystectomy who met the inclusion criteria were included in the study. 3cc bile was aspirated from all patients, this collected bile from gallbladder before cholecystectomy was transported to the laboratory in sterile test-tube. The specimen was evaluated to find out whether it is sterile or has any bacteria present. The types of bacteria are determined and whether the amount of isolate is significant or not. And sensitivity to antibacterial agents against antibiotics was determined.

**Results:** 19 patients showed positive bile culture in which Escherichia coli was the most common isolated bacteria (63.16% among positive bile culture and 15.38% among all patients) and bile was sterile in 59 patients (75.64%). Other organisms isolated were Pseudomonas (3.85%), Klebsiella (2.56%), coagulase negative Staphylococcus and Staphylococcus viridans (1.28%). Positive bile culture was a more common finding (50% of patients were bile culture positive) in patients with acute cholecystitis in this study. Post-operative wound infection is more common (15.79%) in group of patients with isolated organism from bile. There is a strong correlation between bile culture and wound culture (75%).

**Conclusions:** It was found that sensitivity to third- and fourth-generation cephalosporins was higher as compared to aminoglycoside in acute as well as chronic cholecystitis. In this study levofloxacin also shows good sensitivity against isolated organism from bile. Piperacilin and tazobactum also shows good sensitivity against isolated organism from bile and they are more effective against pseudomonas. The resistance to second-generation cephalosporins and aminoglycoside has increased. For preoperative prophylaxis third and fourth-generation cephalosporins and levofloxacin show better promise and may be used as the first line of preoperative prophylaxis in operations for acute and chronic cholecystitis undergoing cholecystectomy.

**Keywords:** Surgery, Cholecystectomy, Bacteriology

## INTRODUCTION

Cholecystectomy is currently a frequently performed operation. The most common reason for a cholecystectomy is gallbladder stones. However, the presence of gallstones within either the gallbladder or biliary tree is associated with the bacterial colonization of the bile. In patients without gallbladder stone disease, previous biliary intervention is associated with high rates of bacteriobilia. Under conditions of normal bile flow, bacteria in the biliary system are of no clinical significance. Upon bile duct obstruction, bacteria proliferate within the stagnant bile while biliary pressure increases. Eventually, the bacteria presumably translocate into the circulation causing a systemic infection. Acute cholangitis spans a continuous clinical spectrum and can progress from a local biliary infection to advanced disease with sepsis and multiple organ dysfunction syndrome. Therefore, it is important to know the microbiological flora of the gallbladder before prophylactic antibiotics are given. In infected bile, the typical pathogens are the Gram-negative enteric aerobes such as Escherichia coli, Klebsiella species and Proteus specie, while Pseudomonas aeruginosa, Bacteroides fragilis and Enterococcus faecalis are less commonly cultured. Prophylactic antibiotics prevent infections even though they do not sterilize bile rates of bactibilia are not reduced by prophylactic antibiotics that achieve bile levels that exceed the minimum inhibitory concentrations of recovered bacteria. Yet, decreased rates of postoperative infection are invariably reported in patients who have received prophylaxis even when rates of bactibilia are similar in treated patients and untreated controls. Prophylaxis would be appropriate according to bacteria isolated from the bile and could prevent postoperative infections. To justify antibiotic prophylaxis against biliary organisms, it requires to be shown that the bile is colonized with bacteria.

### *Aims of study*

- To evaluate the microbiological profile of bile from gall bladder in patients undergoing cholecystectomy.
- To determine the appropriate antibiotic for preoperative prophylaxis in cholecystectomy patients based on the microbiological profile of bile.

## METHODS

This is an observational study carried out at S.S.G Hospital, Baroda between 1st September 2013 to 31st December 2014. The study was under taken after clearance from Scientific Review Committee and Institutional Ethics Committee for Human Research (IECHR) Medical College and SSG Hospital, Baroda. Inclusion criteria were patients undergoing cholecystectomy and patient giving informed consent for study. Patients' age less than 18 year, immunocompromised, patients with any known source of sepsis, patients with history of ascending cholangitis, patients with preoperative diagnosis of empyema of gallbladder, patients who had undergone endoscopic retrograde cholangiopancreaticography (ERCP) and endoscopic sphincterotomy within one week before surgery were excluded from the study. Total 78 patients were studied. Sample size was selected by assuming 26.6% of E. coli isolated from the bile and 10 % fixed allowable error.

Patient's demographic data like name, age, and sex noted. Detailed history was taken with physical examination and investigations were done. Ultrasonography, computed tomography and MRCP were done to confirm the gall bladder pathology before surgery (Figure 1, 2 and 3).

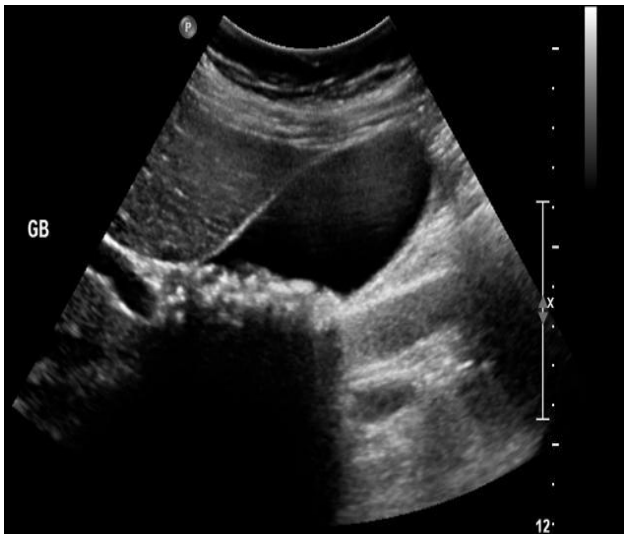


**Figure 1: Ultrasonography image shows multiple echogenic calculi in the gall bladder with dense after shadow.**

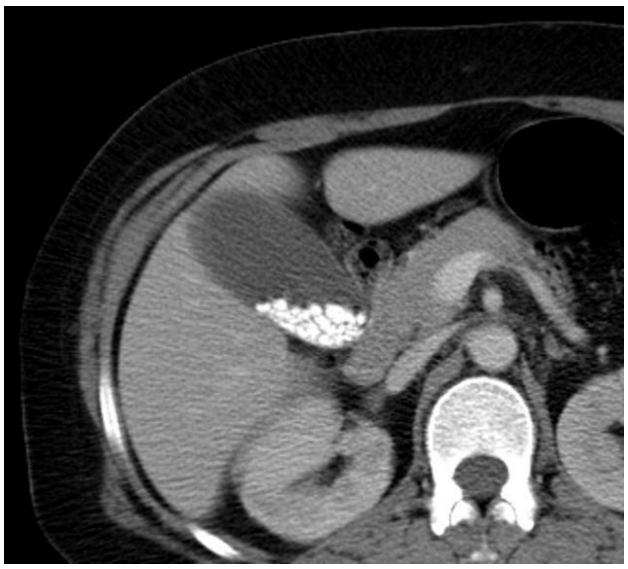

**Figure 2: Axial CT image in the portal venous phase shows multiple hypersende gall bladder calculi.**

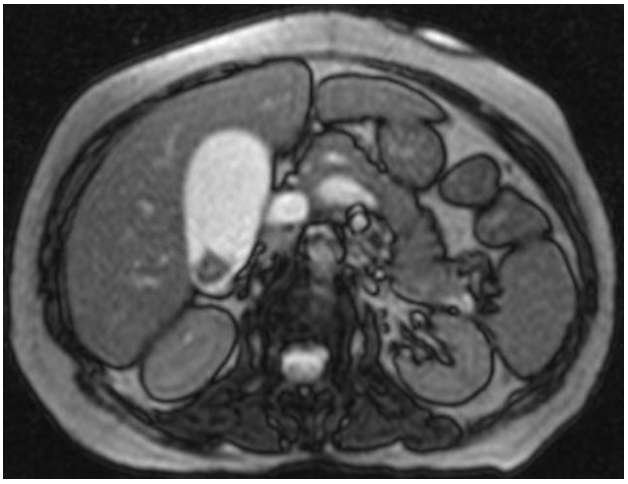

**Figure 3: Axial T2 weighted MR image shows low signal intensity gall bladder calculus.**

Antibiotics were given at time of induction. Name of procedure, date of procedure and any intraoperative complications noted. 3cc bile was aspirated with use of sterile 20 No. spinal needle with 10cc sterile syringe in laparoscopic cholecystectomy (Figure 4, 5 and 6). (After inserting all 3 ports and before starting dissection sample was aspirated) and sterile 10cc syringe with 20 No. needles in open cholecystectomy. This collected bile from gallbladder before cholecystectomy was transported to the laboratory in sterile test-tube. The specimen was evaluated to find out whether it is sterile or has any bacteria present. The types of bacteria are determined and whether the amount of isolate is significant or not. And sensitivity to antibacterial agents determined. For isolation, bile was first examined for gram smear then inoculation of bile for a culture in culture media like brain heart infusion agar, Mac-Conkey agar and blood agar, and then isolated organism identified by biochemical tests. Antibiotic sensitivity testing was performed for the same.

**Figure 4: Aspiration of bile in laparoscopic cholecystectomy with 10cc sterile syringe and 20 No. spinal needle.**

**Figure 5: Aspiration of bile in laparoscopic cholecystectomy with 10cc sterile syringe and 20 No. spinal needle.**

**Figure 6: Sterile test tube.**

## RESULTS

A total of 78 patients admitted to Surgery Department, of Baroda Medical College and S.S.G Hospital, and undergone laparoscopic or open cholecystectomy were included in the study. An evaluation was undertaken to study Bacteriological analysis of bile and sensitivity of isolated organism to antibiotics was determined. Out of these 19 patients whose bile cultures were positive and 59 patients whose bile cultures were negative.

Maximum number of patients belonged to 41-50 years. Organism isolation from bile found maximum in 19-30 years age group. Females were predominated. Organism was isolated in total 19 patients from which 5 patients with acute cholecystitis and 14 patients with chronic cholecystitis. In 59 patients no organism was isolated, from which 5 with acute cholecystitis and 54 with chronic cholecystitis. From 19 patients in which organism isolated; 14 patients underwent laparoscopic cholecystectomy and in 5 patient's laparoscopic cholecystectomy need to be converted in to open cholecystectomy. From 59 patients in which organism were not isolated; 52 patients underwent laparoscopic cholecystectomy, 3 patients with open cholecystectomy and in 4 patient's laparoscopic cholecystectomy need to be converted in to open cholecystectomy. In most of the patients duration of surgery was 1.31-2.30 hours. In 15.38% (i.e. 12 patients) E. coli, 3.85% (i.e. 3 patients) Pseudomonas, 2.56% (i.e. 2 patients) Klebsiella, 1.28% (i.e. 1 patient) coagulase negative Staphylococcus (Figure

7) and in 1.28% (i.e. 1 patient) Staphylococcus viridance was isolated in bacterial culture. In 75.64% (i.e. 59 patients) no organism isolated.

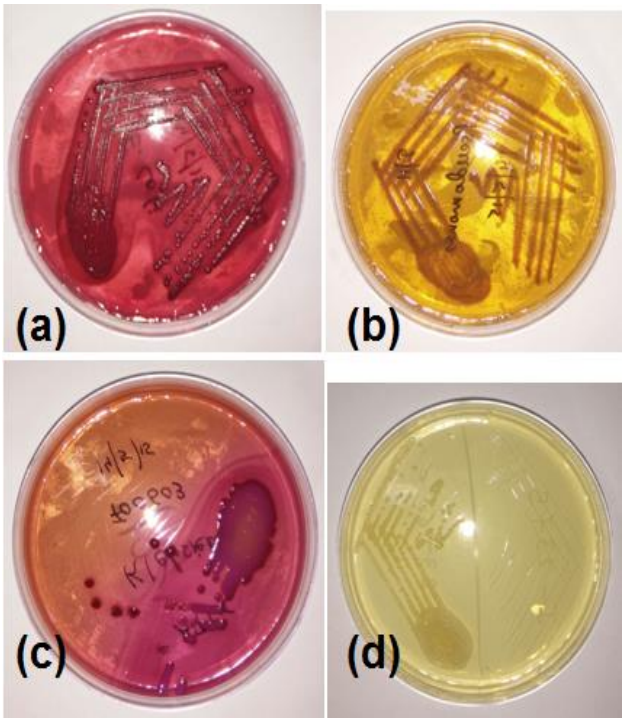


**Figure 7: (a) Mac-Conkey agar shows growth of E. coli (b) Brain heart infusion (BHI) agar shows growth of Pseudomonas (c) Mac-Conkey agar shows growth of Klebsiella (d) Brain heart infusion (BHI) agar coagulase negative Staphylococcus.**

Bacterial sensitivity to the antibiotic result was as mentioned in Table 1. Most of the organism shows sensitivity against cefoparazone, sulbactum, piperacillin, tazobactum, levofloxacillin & meropenem. Multiple antibiotics resistant organisms are tested sensitivity against netilmicin, imipenam, cefepime + tazobactam, aztreonam, cefuroxime, ceftazidime, amoxycillin + clavulinic acid and doxycycline. In one patient, isolated organism from bile was E. coli which was multiresistant to all antibiotics except imipenam, cefepime + tazobactam.

**Table 1: Sensitivity patterns of common antibiotics used.**

| Antibiotics | Sensitive | Resistant |
|---|---|---|
| Amikacine | 11 | 05 |
| Gentamycin | 07 | 10 |
| Cefotaxime | 06 | 09 |
| Cefipime | 10 | 06 |
| Cefoparazone + Sulbactum | 16 | 00 |
| Piperacilin + Tazobactum | 16 | 00 |
| Levofloxacilin | 13 | 00 |
| Meropenem | 16 | 00 |

Post-operative wound infection is more common (15.79%) in group of patients with isolated organism from bile. 4 patients was developed post-operative wound infection. From which E. coli, Enterococcus species, Klebsiella, and no organism was isolated in 25% each. It was same as the post-operative wound organism. There is a strong correlation between bile culture and wound culture (75%).

## DISCUSSION

Bile in the gallbladder or bile ducts in the absence of gallstones or any other biliary tract disease is normally sterile. In the presence of gallstones or biliary obstruction, the prevalence of bactibilia increases. The percentage of positive gallbladder bile cultures among patients with symptomatic gallstones and chronic cholecystitis ranges from 11% to 30%. Positive bile cultures are significantly more common in elderly (>60 years) patients with symptomatic gallstones than in younger patients (45% versus 16%).[1,2] Gram-negative aerobes are the organisms most frequently isolated from bile in patients with symptomatic gallstones, acute cholecystitis or cholangitis. Extensive literature characterizes the epidemiology and risk factors for postoperative wound infections after gallbladder surgery.[3,4] These studies document a strong association between the presence of bacteria in bile cultures taken at surgery and the occurrence of subsequent infection.[5-7]

Prophylactic antibiotics prevent infections even though they do not sterilize bile[8,9] Rates of bactibilia are not reduced by prophylactic antibiotics that achieve bile levels that exceed the minimum inhibitory concentrations of recovered bacteria.[10] Yet, decreased rates of postoperative infection are invariably reported in patients who have received prophylaxis even when rates of bactibilia are similar in treated patients and untreated controls.[11] In most studies, infections develop in only 5-15% of patients with positive bile cultures who received prophylaxis.[12,13]

In this study a total of 78 patients (54 female and 24 male) who met the inclusion criteria were included both open as well as laparoscopic cholecystectomy. Numbers of Patients with positive bile cultures were 19 (24.36%) and those with negative culture were 59 (75.64%). According to the Suri et al. study[14] was conducted in 150 consecutive patients (113 females and 37 males Female:Male=3:1) and included both open as well as laparoscopic cholecystectomy. The age pattern for patients presenting for cholecystectomy showed that most of the patients (79; 52.67%) were belonging to the 3rd and 4th decades of life. In Alaattin Öztürk et al. study,[15] a total of 114 patients were included in this study. Ages ranged from 17 to 86 years with a mean age of 48.6 ± 16.8. 81 patients (71.1%) were women and 33 (28.9%) were men. (Female:Male=2.5:1). In this study Majority of patients found in age group between 41-50 years (23 patients; 29.49%). Organism found maximum in 19-30 years age

group. Majority of the patients in both groups were females (54 females and 24 males Female:Male=2.3:1).

In Suri et al. study,[14] the highest incidence of positive cultures was noted in patients with acute cholecystitis (40%) Although this difference was statistically not significant due to P value of 0.1266. In Alaattin Öztürk et al. study,[15] the highest incidence of positive cultures was noted in patients with chronic cholecystitis (66.7%). In acute cholecystitis incidence of positive cultures was (20%) and third group acute cholecystitis with choledocholithiasis incidence were 3 patients out of 15 (13.3%). Among this group difference was statistically significant because P value is less than 0.005 (P value was=0.003). In this study positive bile culture was a more common finding (50% of patients were bile culture positive) in patients with acute cholecystitis. In the vast majority of patients with chronic cholecystitis, the bile was sterile (only 20.59% of patients were bile culture positive). Although this difference was statistically not significant (P value - 0.1034).

In Acharya Suri et al.;[14] on gram staining, no organism was found microscopically in the bile of 24 (82.6%) patients. Later on, it was confirmed that such bile was sterile because no growth appeared on culture plates, both aerobic and anaerobic. In the present study only aerobes were cultivated. Escherichia coli (53.84%) was one of the most common isolated bacteria followed by Pseudomonas aeruginosa (26.92%) and Staphylococcus aureus (19.23%). In none of the cultures Streptococcus, Clostridium or Klebsiella was present. In our study, 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%). These findings are similar to as observed in other studies.[16]

In Acharya Suri et al.;[14] the sensitivity of the organisms grown in our analysis of 26 out of 150 patients was tested against cefuroxime, cefoperazone and cefepime, and it was found that sensitivity to third- and fourth-generation cephalosporins was higher as compared to second-generation cephalosporins in acute as well as chronic cholecystitis. The resistance to second-generation cephalosporins has increased while third- and fourth-generation cephalosporins show better promise and may be used as the first line of preoperative prophylaxis in operations for cholecystectomy.[16] In our study, sensitivity of the organisms grown in our analysis of 19 out of 78 patients were tested against amikacine, gentamycin, cefotaxime, cefipime, meropenem, cefoparazone + sulbactum, piperacilin + tazobactum, levofloxacilin and it was found that sensitivity to third- and fourth-generation cephalosporins (cefoparazone, cefepime) was higher as compared to aminoglycoside in acute as well as chronic cholecystitis. Levofloxacin also shows good sensitivity against isolated organism from bile. Piperacilin and tazobactum also shows good sensitivity against isolated organism from bile and they are more effective against pseudomonas. Majority of the organisms were sensitive to third generation cephalosporins and one patient had multi resistant organisms. The resistance to second-generation cephalosporins and aminoglycoside has increased while third generation, fourth generation cephalosporins and levofloxacin show better promise and may be used as the first line of preoperative prophylaxis in operations of cholecystectomy.

Van Leeuren PA et al.[17] study, total 840 patients from which 138 patients showed positive bile culture (16.4%) and 72 patients showed wound infection (9%). Bile culture showed E. coli was most common organism isolated (36%). But there was no correlation between positive bile culture and subsequent wound culture.

Valazquez-Mendoza JD et al.[18] study; total 80 patients study, 40 patients with bile culture positive and 40 patients with wound culture positive. There was no statistically significant difference when comparing surgical site infection in both groups. In our study, 4 patients showed wound infection (5.1%) and 3 patients (out of 4 patients with wound infection) showed micro-organism in culture (3.9%) which was similar with micro-organism isolated in bile culture of same patients. All 3 patients with wound infection had their bile culture positive for same micro-organism. Clinically there was strong correlation between bile culture and wound culture. Statistically on application of rank correlation P value 0.0151. This shows there was a statistically significant correlation between bile culture and same wound culture.

Limitation of the study was small study sample size. In order to draw valid conclusion regarding type of organism isolated from bile and sensitivity pattern to antibiotics a larger sample size is required.

## CONCLUSION

In majority of patients the bile was sterile. Only 19 (24.4%) patients had positive bile culture. Escherichia coli was the most common isolated bacteria among positive bile culture group (63.16%) and 15.38% among all patients. The third generation, fourth-generation cephalosporins and levofloxacin show better promise and may be used as the first line of preoperative prophylaxis in operations for acute and chronic cholecystitis patients undergoing cholecytectomy. Post-operative wound infection is more common (15.79%) in group of patients with isolated organism from bile.

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