



CLINICAL ASPECTS OF BILE CULTURE IN PATIENTS UNDERGOING LAPAROSCOPIC CHOLECYSTECTOMY

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ABSTRACT

Bile 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. Bactibilia is one of the most important causes of acute cholecystitis. The selection of appropriate antibiotics largely depends on knowledge about the microbial flora of the biliary system. The aim of this study was to evaluate the incidence of biliary microflora and prepare an antibiogram for gall bladder pathologies. **Methods:** It was a prospective study involving 60 patients. Those who met the inclusion criteria were selected, 5 millilitres of bile removed from their gallbladders before the cholecystectomy, and the collected bile was transported to the laboratory in sterile test tubes. The samples were cultured and tested for antibiotic sensitivity if cultures were positive. **Results:** In our study, E. coli was the most common organism isolated. The results were 53% no growth followed by 15% Escherichia coli, 11.7% Klebsiella, 8.3% Proteus vulgaris, 6.7% Enterococcus, 3.3% Salmonella and 1.7% shigella. Noone developed postoperative wound infection regardless of culture status. **Conclusion:** E. coli was the bacteria that was isolated from bile culture the most frequently. Ceftriaxone and Piperacillin-Tazobactam demonstrated the greatest degree of effectiveness against these pathogens. Ceftriaxone and/or PIPTAZ need to be a part of the empirical regime in order to help reduce the morbidity associated with symptomatic cholelithiasis.

KEYWORDS : cholecystectomy, antibiotics, bactobilia

INTRODUCTION

The incidence of gallstone illnesses is on the rise, and cholecystectomy is rapidly becoming the elective abdominal procedure that is performed most frequently these days. As technology related to laparoscopy has improved, laparoscopic cholecystectomy has emerged as the method of choice for removing gallbladders. In addition to it, however, still open cholecystectomies are being carried out. In comparison to open cholecystectomy, the risk of post-operative complications has been greatly mitigated by the development of laparoscopic cholecystectomy; however, there is still a possibility of post-operative bile leakage and infection at the surgical site. Since bile is thought to be sterile, cholecystectomy is typically seen as a relatively risk-free surgical treatment. The presence of bacteria in bile, on the other hand, is a risk factor that can raise the likelihood of unfavourable outcomes both before and after surgery. Bactibilia is a risk factor for poor operative outcomes, and if we have the right knowledge about the biliary microflora and its antibiotic sensitivity, we can decrease the likelihood of these poor outcomes by using appropriate prophylactic antibiotics. Bactibilia is a factor that contributes to poor operative outcomes. Even with laparoscopic cholecystectomy, there is still a possibility of bile leakage or spilling, which can lead to an increased risk of undesirable intra- and post operative outcomes. The presence of bacteria in bile heightens the danger, which in turn leads to an increase in post-operative morbidity and a lengthier stay in the hospital. Therefore, the purpose of this study is to investigate the biliary microflora in order to determine which organisms are the most common in the areas surrounding our medical college, whether they are single strain or multi strain, and which antibiotics are sensitive to and which are resistant to these organisms. After the analysis is complete, an antibiogram can be developed for gall bladder diseases, which will ultimately result in a reduction in morbidities.

The primary aim of this study was to identify organisms present in bile of cholecystectomy patients and see for antibiotic sensitivity and prepare an antibiogram for gall bladder pathologies in a tertiary care hospital in south India.

MATERIALS AND METHODS

It was a prospective observational study conducted in the Department of General Surgery, Kilpauk Medical College & Hospital and Govt. Royapettah Hospital for a duration of 6 months April, 2023 – September, 2023. Sample size of 60 was calculated using the culture-positivity as primary outcome measure. Institutional ethical committee approval was obtained and written informed consent was obtained from participants. Inclusion criteria were patients above the age of 18 and all patients undergoing laparoscopic/open cholecystectomy. All patients below the age of 18, pregnant women, immuno-compromised persons, and those who were not willing for participation. Preoperative diagnosis included all cases of cholecystectomy including acute and chronic cholecystitis, symptomatic cholelithiasis and post ERCP and stenting patients. 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.

Prophylactic antibiotics with second-generation cephalosporin were administered once 30minutes before surgery. Oral antibiotics were not used. Under aseptic precautions, intra operatively, 5ml bile was aspirated with use of sterile 20 no. spinal needle with 10ml sterile syringe in laparoscopic cholecystectomy after inserting ports and before starting dissection and sterile 10cc syringe with 20 no. needles in open cholecystectomy. This collected bile before cholecystectomy was transported to the microbiology laboratory in sterile test-tube. The specimens were evaluated to find out whether it was sterile or had any bacteria present, to determine the types of bacteria and whether the amount of isolate was significant or not. Sensitivity to antibacterial agents were determined. For isolation, Gram staining followed by inoculation into brain heart infusion agar, Mac-Conkey agar and blood agar were used and incubated at 37°C in ambient air. Isolated organisms were identified using biochemical tests. Antibiotic sensitivity testing was performed for the same. Antimicrobial susceptibility testing performed using Kirby-Bauer's disc

diffusion method on Muller-Hinton agar using oxoid antibiotic discs. All collected information was entered into a database as either continuous (quantitative data) or categorical (quantitative data) variables.

RESULTS

A total of 60 patients who got admitted for laparoscopic or open cholecystectomy were included in the study and bile samples were collected from all 60 patients. In our study, majority of the patients were females with a frequency of 23.3% males and 76.7% females. About 13.3% of patients belonged to the age group of 20-30 years, 25% 31-40 years, 13.3% 41-50 years, 25% 51-60 years and 23.3% 61-70 years. Around 26.7% are vegetarians and 73.3% are on mixed diet.

Around 41.6% had nausea/vomiting, 31.6% had dyspepsia, 40% had fever. Almost all patients had abdominal pain. Around 38.3% were having cholesterol/yellow stones and 61.7% had pigment/black stones.

Bacteriological analysis of bile and sensitivity of individual isolated organism to antibiotics determined. And results obtained from culture reports were analyzed. It was found out that, out of the 60, 28 patients had bile cultures positive and in 32 patients, bile cultures were negative. Among the 28 culture positive patients, 15% (9 patients) *Escherichia coli*, 11.7% (7 patients) *Klebsiella*, 8.3% (5 patients) *Proteus vulgaris*, 6.7% (4 patients) *Enterococcus*, 3.3% (2 patients) *Salmonella* and 1.7% (1 patient) *Shigella* were isolated.

Escherichia coli showed high sensitivity towards ceftriaxone 88.9%, 77.8% sensitivity towards Piptaz, 44.4% towards ciprofloxacin and 22.2% towards amoxicillin. *Escherichia coli* showed high resistance towards amoxicillin 77.8%, 55.6% resistance towards ciprofloxacin and 11.1% resistance towards Amikacin and ceftriaxone respectively. *Enterococcus* showed high sensitivity towards piptaz 75%, 75% towards ciprofloxacin, 50% towards amoxicillin and 25% towards ceftriaxone. *Enterococcus* showed high resistance towards ceftriaxone 75%, 50% towards amoxicillin, 25% towards ciprofloxacin and Meropenam respectively. *Klebsiella* were 71.4% sensitive towards ceftriaxone, 57.1% sensitive to Piptaz, 57.1% sensitive towards ciprofloxacin, 75% sensitive towards amoxicillin and 14.3% towards Amikacin. *Klebsiella* was highly resistant towards amoxicillin 57.1%, 28.6% towards Meropenam, Ceftriaxone and Ciprofloxacin respectively. *Proteus vulgaris* was highly sensitive towards ceftriaxone 100%, 60% towards Piptaz, 60% towards ciprofloxacin and 40% towards amikacin. *Proteus vulgaris* showed marked resistance towards amoxicillin 100% and 40% towards ciprofloxacin and 20% towards Piptaz. *Salmonella* was highly sensitive towards ceftriaxone, 50% towards Piptaz, 50% ciprofloxacin and 50% Meropenam. *Salmonella* resistance 50% towards Piptaz, 50% to ciprofloxacin and 100% to amoxicillin. *Shigella* was sensitive towards Ceftriaxone, Ciprofloxacin and Amoxicillin. *Shigella* was resistant towards Piptaz, Ciprofloxacin and Amoxicillin.

No patients developed postoperative SSI.

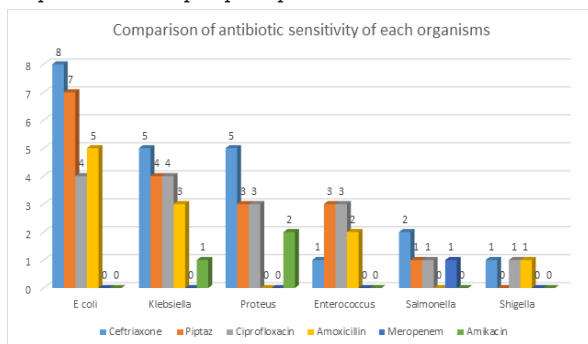


Figure 1: Chart Showing How Each Bacteria Responded To Individual Antibiotics.

For eg, among 9 positive *E. coli* cultures, 8 were sensitive to Ceftriaxone while one was not; 7 cultures showed sensitivity to Piptaz; 4 showed sensitivity to Ciprofloxacin while 5 was resistant; 5 were sensitive and 4 were resistant to Amoxicillin and all 9 were resistant to Meropenem and Amikacin.

DISCUSSION

Bactibilia is one of the most important causes of acute cholecystitis, and thus is the main target of therapy. Positive bile culture rates range from 9% to 42% in electively resected gallbladders; in cases of acute cholecystitis, its incidence is from 35% to 65%.^(2,5-7)

A total of 23.3% are males and 76.7% are females. In a study that was conducted by Thapa SB. and colleagues⁽¹⁸⁾, the total number of patients who presented with symptoms of cholelithiasis was 259. It was found that there were 64 male patients (24.71 percent) and 195 female patients (75.29 percent) respectively, with a male to female patient ratio of 1:3, which was in agreement with our study. According to the available research, gallstone disease is more prevalent among obese, fertile women over the age of forty.

Analysis of the patients' eating habits was performed because of the significance of their participation in the development of gallstones in the pathogenesis of the disease. In our study around 26.7% are vegetarians and 73.3% are on mixed diet. The most common clinical presentation among the cases studied was abdominal pain, all the cases studied presented with abdominal pain (100%). The findings of our research were consistent with those found in the Multicentre Italian Study of Cholelithiasis. Laparoscopic cholecystectomy was the most prevalent mode of surgery that was performed for our patients, and it was performed on all of our patients.

In our study around 38.3% were having Cholesterol/Yellow stones and 61.7% had Pigment/Black stones. In 32 of the cases, bile culture reports showed no evidence of the growth of any organism (53 percent). The percentage of bacteria that grew in bile cultures ranged from 16 to 54 percent, according to the findings of various investigations.^(5,19-21)

In our study there were about 53% no growth followed by 15% *Escherichia coli*, 11.7% *Klebsiella*, 8.3% *Proteus vulgaris*, 6.7% *Enterococcus*, 3.3% *Salmonella* and 1.7% *shigella*. Our findings were quite similar to that of a study done by Abeyasuriya et al⁽²²⁾ in which the researchers found that bacterial isolates were considerably more abundant in bile that included pigment stones than in bile that contained cholesterol stones.

According to the findings of a study conducted by Masahisa Tabata et al.⁽²³⁾, the incidence of bacteria was quite high in instances involving bile pigment calcium stones as well as combination stones. In contrast, it was discovered that the incidence of black stone cases was far lower than expected.

A total of 104 bile samples were evaluated in the research carried out by Capoor et al.⁽²⁴⁾, and bacteria were found in 37 of those samples (35.6 percent). The most common species isolated were *Escherichia coli* (11, 29.7 percent), *Klebsiella pneumoniae* (10, 27 percent), *Citrobacter freundii* (3, 8.1 percent), *Salmonella enterica* serovar typhi (3, 8.1 percent), *Pseudomonas aeruginosa* (2, 5.4 percent), *Acinetobacter* spp. (1, 2.7 percent), *Candida krusei* (1, 2.7 percent), *Staphylococcus aureus* (1, 2.7 percent). Infection of *P. aeruginosa* by several microorganisms, including *K. pneumoniae* was found in four different patients (3.8 percent). A total of 125 bile samples and 25 gall stones were analysed

for the presence of aerobic and anaerobic microorganisms as part of a study that was carried out by Ballal et al.⁽²⁵⁾. 88 out of 125 patients (70.4 percent) had positive bile culture results for the presence of germs. According to the findings of the study on the bacterial flora, *Escherichia coli* was the most typical organism to be found in bile as well as in gall stones. This bacterium may be found in clinical specimens either on its own or in conjunction with other types of organisms. *Salmonella typhi* was the first organism to be isolated from the two bile samples, followed by *Klebsiella*. The highest number of isolates, 34, were found in people aged 51 to 60 years old (45.4 percent).

In contrast to studies conducted by Dhiel et al.⁽²⁶⁾ and Thapa SB. et al.⁽¹⁸⁾, in which *Pseudomonas* was shown to be the most frequent organism in bile culture, our research found that this was not the case.

Ozturk et al.⁽²⁷⁾ conducted a study in which they asked 114 patients who had had cholecystectomy for a variety of reasons to participate in the study. There was evidence of bacterial growth in the bile cultures taken from 15 patients (13.1 percent). *Enterococcus* spp (4 patients, 26.6 percent), *Escherichia coli* (3 patients, 20 percent), and *Enterobacter* spp were the types of bacteria that were found to be the most frequently isolated (3 patients, 20 percent). Patients who suffered from acute cholecystitis in addition to cholelithiasis had the highest rate of positive bile culture results (3 patients, 100 percent). The rate of bacterial growth in the bile culture was highest in patients who were over the age of 60 (10 patients, 27 percent), as well as in patients who had a concurrent illness (9 patients, 23.6 percent). Only one patient was found to have an infection at the surgical site after the procedure, while patients who had a positive bile culture did not have any infections at the surgical site.

In our study *Escherichia coli* showed high sensitivity towards ceftriaxone 88.9%, 77.8% sensitivity towards Piptaz, 44.4% to ciprofloxacin and 22.2% to amoxicillin. *Escherichia coli* showed high resistance towards amoxicillin 77.8%, 55.6% resistance towards ciprofloxacin and 11.1% resistance to amikacin and ceftriaxone. *Enterococci* showed high sensitivity towards Piptaz (75%), 75% towards ciprofloxacin and 50% towards amoxicillin. *Enterococcus* showed high resistance towards ceftriaxone 75%. *Klebsiella* were 71.4% sensitive towards ceftriaxone, 57.1% sensitive to Piptaz 57.1% sensitive to ciprofloxacin, 75% sensitive to amoxicillin and 14.3% to amikacin. *Klebsiella* was found to be resistant to Amikacin. *Proteus vulgaris* was highly sensitive towards ceftriaxone 100%, 60% towards Piptaz, 60% towards ciprofloxacin and 40% to amikacin. *Proteus vulgaris* was highly resistance to amoxicillin 100% and 40% to ciprofloxacin. *Salmonella* was highly sensitive to ceftriaxone, 50% to Piptaz, 50% ciprofloxacin and 50% meropenam. *Salmonella* showed a resistance level of 50% to Piptaz, 50% to ciprofloxacin and 100% to amoxicillin. *Shigella* was sensitive to ceftriaxone, ciprofloxacin and amoxicillin. *Shigella* was resistant to Piptaz.

Although surgical intervention is still the basis of therapy for acute cholecystitis and its complications, a period of hospitalisation is required prior to elective or emergency cholecystectomy. This is because of the risk of complications during surgery. In the current investigation, the empirical antibiotics were administered in accordance with the accepted guidelines and they were altered as the findings of the culture and sensitivity tests were available. Antibiotic-treated patients have a lower risk of developing postoperative complications related to wound infection, such as abscess formation or sepsis. In a nutshell, the use of non-steroidal anti-inflammatory medicines, more commonly referred to as NSAIDs, is indicated for the treatment of mild cases of biliary

colic in order to stop the progression of inflammation. For infections that are mild in severity, it is best to use drugs that have a narrow spectrum of activity, such as Piptaz or ciprofloxacin combined with metronidazole. Combination medicines or carbapenem are typically indicated for treating severe infections. This latter condition required not only oral intake to be discontinued but also hydration and electrolyte correction.

It would appear that a history of past and recurring hospitalisation, prolonged hospital stay, and widespread usage of broad range antibiotics have all contributed to the selective survival and growth of antibiotic-resistant organisms.⁽²⁸⁾ When it comes to prescription medications, therefore, antibacterial activity against possible causal organisms, the severity of the cholecystitis, and the local susceptibility pattern are all factors that need to be taken into consideration. Previous research has found that the antibiotic combinations piperacillin-tazobactam and meropenem produce the best results, with quinolones being the preferred choice for Gram-negative isolates and vancomycin being the preferred choice for Gram-positive infections.⁽²⁹⁾ When it comes to the prescription of medications, antibacterial activity against suspected causal organisms, the severity of the cholecystitis, and the local susceptibility pattern are all factors that need to be taken into consideration.

CONCLUSION

E. coli was the bacteria that was isolated from bile culture the most frequently, followed by *enterococcus*, *klebsiella*, *proteus vulgaris*, *salmonella*, and *shigella*. Ceftriaxone and Piptaz demonstrated the greatest degree of effectiveness against these pathogens. Antibiotics used in the treatment of symptomatic gall stone disease must cover these prevalent microorganisms in order to be considered empirical treatments. Ceftriaxone and/or Piptaz need to be a part of the empirical regime in order to help reduce the morbidity associated with symptomatic cholelithiasis.

Limitations Of The Study: small study group.

Conflicts Of Interest: Nil

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