



PREVALENCE OF MICROORGANISMS IN GALL BLADDER ASPIRATE IN PATIENTS UNDERGOING LAPAROSCOPIC/OPEN CHOLECYSTECTOMY FOR CHRONIC CALCULUS CHOLECYSTITIS IN A TERTIARY CARE CENTRE

Dr Rishi Ranjan Shrivastava

Senior Resident (bond under W.B.U.H.S.), Department of General Surgery, Baruipur Subdivisional Hospital and Super Speciality Hospital, South 24 Parganas, West Bengal.

Dr Pratip Sengupta

Associate Professor, Department of General Surgery, Ramakrishna Mission Seva Pratishthan, Vivekananda Institute of Medical Sciences, Kolkata, West Bengal.

Dr Suniti Kumar Hajra

Assistant Professor, Department of General Surgery, Ramakrishna Mission Seva Pratishthan, Vivekananda Institute of Medical Sciences, Kolkata, West Bengal.

Dr Debayan Chowdhury*

3rd year DNB Post Graduate Trainee, Ramakrishna Mission Seva Pratishthan, Vivekananda Institute of Medical Sciences, Kolkata, West Bengal.
*Corresponding Author

ABSTRACT

Background: Gallstone disease remains one of the most common medical problems leading to surgical intervention. The presence of gallstones within either the gallbladder or biliary tree is associated with the bacterial colonization of the bile. In the vast majority of cases, infection is due to bacteria originating from the biliary tract. The incidence and type of bacteria involved and their susceptibility to antibiotics can be accurately predicted by bacteriological examination of the bile and a suitable antibiotic regimen can then be selected to minimize post-surgical infections. Therefore, it is important to know the microbiological flora of the gallbladder before prophylactic antibiotics are given.

Materials and Methods: Study design: A descriptive study. **Study area:** Ramakrishna Mission Seva Pratishthan Hospital, Kolkata. from October 2017 to April 2019 (18 months). **Sample size:** 300 patients admitted in the Department of General Surgery undergoing Laparoscopic/Open Cholecystectomy. **Inclusion criteria:** Patients diagnosed with Chronic Calculous Cholecystitis belonging to any age or sex. **Exclusion criteria:** Patients with acute cholecystitis, empyema gallbladder, mucocele of gallbladder, gallstones along with bile duct stones. **Methodology:** Intraoperatively, 2ml bile from gallbladder was aspirated and sent for microbiological analysis. Each specimen was inoculated on two blood agar plates and one MacConkey agar plate. Growth was confirmed from colony characteristics, by Gram's staining, biochemical reactions and any special tests required confirming the particular bacterium. Antibiotic sensitivity was done by Kirby-Bauer method.

Results: Bile Culture was positive in 39(13%) patients, of which majority were Females(61.5%) and majority were in the 61-80 age group for both. The most common organism cultured in Bile was E.coli(59%), followed by Klebsiella (25.6%). Majority were sensitive to Cefuroxime (28.2%), followed by Ciprofloxacin (20.5%). Metronidazole was found sensitive in 12.8% cases whereas, Levofloxacin, Meropenem and Piperacillin-Tazobactam were sensitive in 10.3%, 12.8 and 5.1% cases. 3 cases (7.7%) were found to contain microorganisms resistant to commonly used antibiotics.

Conclusion: Biliary cultures were positive in 13% of cases with enterobacteria being the most frequent isolate.

KEYWORDS : Gallstone Disease, Microorganisms, Bile Aspirate, Bile Culture, Antibiotic Sensitivity.

INTRODUCTION

Cholelithiasis is one of the most common disorders affecting the gastrointestinal tract and is an important cause of morbidity. In India, it is more common in North India as compared to South India. Similarly, incidence in Eastern India is higher than in Western India¹.

The presence of bacteria in bile at the time of surgery or invasive diagnostic procedures predisposes to septic complications like septicemia, liver abscess, hepatic and renal failure, endotoxemia and disseminated intravascular coagulation and hence the judicious use of prophylactic antibiotics reduce morbidity and mortality due to infection².

The biliary tract is usually sterile; however, if cholelithiasis occurs, different microbes might be identified in and/or cultured from the bile or gallbladder wall³. Infective factor seems to be a major cause of formation of gallstones. Moynihan's aphorism that "gall stone is a tomb stone erected in the memory of the organism with in it" is true today⁴.

The various concepts of pathogenesis of infection of biliary tract are based on four possible routes:

- 1) Hematogenous infection (via hepatic artery or portal vein).
- 2) Descending infection from the liver (Enterohepatic route).
- 3) Ascending infection from duodenum up to the CBD.

- 4) Spreading infection via lymphatics through the wall of the gallbladder².

Ascending infection from the duodenum is thought to be the primary mechanism by which bacteria enter the bile³.

Organisms responsible for biliary tract infection are bacteria E.Coli, Streptococcus faecalis, Klebsiella, Pseudomonas aeruginosa, fungi and ova of parasites- ascariis lumbricoides and Clonorchis sinensis². Most common infection is due to E. coli and typhoid bacilli. These organisms reach the liver by way of portal vein and are then transported via the bile to the bile ducts and gallbladder².

The selection of antibiotics for the treatment of active infection and for the prophylaxis of post-surgical sepsis is dependent upon the nature and sensitivity of the pathogens involved. In the vast majority of cases, infection is due to bacteria originating from the biliary tract. The incidence and type of bacteria involved and their susceptibility to antibiotics can be accurately predicted by bacteriological examination of the bile and a suitable antibiotic regimen can then be selected to minimize post-surgical infections.

Various studies have been done to study the bacterial flora of bile and biliary tract but on no other subject such diversity of opinion exists as on the nature of the gallbladder infection.

The predominant bacterium according to AlHarbi et al⁶ is E.Coli while Wayne and Whelan⁷ reported Klebsiella as predominant organism in the biliary tract. Certain anaerobic and aerobic bacteria have also been found². Hence the various studies done on bacterial flora of biliary tract present a confusing picture. This study aims at evaluating the bacteriological profile, both aerobic and anaerobic of patients undergoing cholecystectomy and to proceed accordingly.

AIM AND OBJECTIVES

- 1) To find out the prevalence of microorganisms in Gall Bladder aspirate in patients undergoing Laparoscopic/Open Cholecystectomy for Chronic Calculus Cholecystitis.
- 2) To find out the drug sensitivity pattern if culture is found positive for bacterial growth.
- 3) To assess the need for prophylactic antibiotics for Chronic Calculous Cholecystitis, which are commonly given in our hospital, and incidence of bactibilia in patients which receive prophylactic antibiotics.

MATERIALS AND METHODS

Study design: A descriptive study.

Study area: Ramakrishna Mission Seva Pratishthan Hospital, Kolkata, West Bengal.

Study period: October 2017 to April 2019 (18 months).

Sample size: 300 patients admitted in the Department of General Surgery undergoing Laparoscopic/Open Cholecystectomy.

Inclusion Criteria:

Patients diagnosed with Chronic Calculous Cholecystitis belonging to any age or sex.

Exclusion Criteria:

Patients with:

- 1) Acute cholecystitis
- 2) Acute acalculous cholecystitis
- 3) Empyema gallbladder
- 4) Mucocele of gallbladder
- 5) Gallstones along with Bile duct stones.

Statistical Analysis:

- 1) Categorical variables are expressed as Number of patients and percentage of patients and compared across the groups using Pearson's Chi Square test for Independence of Attributes/ Fisher's Exact Test as appropriate.
- 2) The statistical software SPSS version 20 has been used for the analysis.
- 3) An alpha level of 5% has been taken, i.e., if any p value is less than 0.05 it has been considered as significant.

METHODOLOGY:

Important operative findings like adhesions at Calot's triangle, or Mirizzi Syndrome, or a difficult laparoscopic cholecystectomy being converted to open, were recorded.

Bacteriological Study:

Intraoperatively, 2ml bile from gallbladder was aspirated with a sterile syringe before manipulation of bile duct. Within one hour of collection, bile was sent to the department of microbiology for processing. Each specimen was inoculated on two blood agar plates and one MacConkey agar plate. One blood agar and MacConkey agar was incubated aerobically at 37°C for 24 hours and other blood agar plate with haemin and menadione incubated anaerobically at 37°C for 48 hours in anaerobic jar to see the aerobic and anaerobic growth respectively. All the samples were subjected to smear examination and culture for both aerobic and anaerobic organisms. Smears were prepared from both specimens and after Gram's staining, were examined for presence of gram

negative and positive bacteria. Culture plates were examined after overnight incubation for presence of growth. Growth was confirmed from colony characteristics, by Gram's staining, biochemical reactions and any special tests required confirming the particular bacterium.

Aerobic Bacteria:

Gram Positive:

- 1) *Staph epidermidis*: On blood agar, colonies are small, circular, smooth with entire margins, butyrous consistency, white in colour and non-haemolytic. Growth confirmed by no haemolysis on blood agar, white colour colonies and negative coagulase test.
- 2) *Staph aureus*: On blood agar, colonies are smooth, golden yellow in colour, low convex, glistening, densely opaque, butyrous consistency and surrounded by a narrow zone of haemolysis. Growth confirmed by haemolysis on blood agar, golden yellow colour and positive coagulase test.
- 3) *Strep faecalis*: On MacConkey agar, colonies are small (0.5 to 1mm), usually magenta coloured and non-haemolytic. Growth confirmed by positive mannitol test and positive heat test.

Gram Negative:

- 1) *E.Coli*: On MacConkey agar, colonies are large (2-3mm), circular, low convex, red or pink in colour and opaque. Growth confirmed by biochemical reactions (indole test positive, methyl red test positive).
- 2) *Klebsiella pneumonia*: On MacConkey agar, colonies are large mucoid and red. Growth confirmed by biochemical reactions (string test positive).
- 3) *Pseudomonas aeruginosa*: On blood agar, colonies are large (2-3mm), smooth, translucent, irregularly round with a characteristic fruity odour. Growth confirmed by production of bluish green pigment (pyocyanin) and oxidase test positive.
- 4) *Proteus*: Culture emits characteristic putrefactive (fishy) odour and possess the ability to swarm (spread on solid media). Growth confirmed by positive phenylalanine deaminase test and positive urease test.
- 5) *Salmonella typhi*: On MacConkey agar, colonies are pale yellow or colourless due to absence of lactose fermentation. Growth confirmed by positive reaction for H₂S in triple sugar iron agar.

Anaerobic Bacteria:

Gram Positive:

Anaerobic cocci - *Peptococcus*: On blood agar, colonies are black in colour on prolonged incubation. Growth is confirmed by negative Catalase test and indole test negative.

Gram Negative:

Bacteroides fragilis: On blood agar, colonies are 1-2mm, smooth, low convex, translucent or light grey with no haemolysis. Growth is confirmed by positive indole test.

After the bacteria were isolated, we performed antibiotic sensitivity tests by Kirby-Bauer method. The applied antibiotic disks were Ampicillin, Cefuroxime, Ciprofloxacin, Levofloxacin, Meropenem, Metronidazole, Piperacillin-tazobactam.

RESULTS

In our study, of all the patients suffering from chronic calculous cholecystitis, 9(3%) patients were less than 20 years age, 113(37.6%) patients between 21-40 years, 127(42.3%) between 41-60 years, 49(16.3%) between 61-80 years and 2(0.6%) patients were more than 80 years age.

217(72.3%) patients were females and 83(27.8%) were males.

31(10.4%) patients had Type 2 Diabetes mellitus, 36(12%) had Hypertension, 11(3.7%) had Chronic obstructive pulmonary disease, 4(1.3%) had Coronary artery disease, 2(0.7%) had Hyperlipidaemia, 11(3.7%) had Hypothyroidism, 1(0.3%) had Prostatomegaly while 242(80.9%) patients had no comorbidities.

Out of 300 patients in our study, Bile Culture was positive in 39(13%) patients, of which 24 were females and 15 were males and majority were in the 61-80 age group for both.

Table 1: Age group of females and males with Bile Culture positive.

Age Group	Females with Bile Culture Positive	Percentage	Males with Bile Culture Positive	Percentage
< =20	0	0.0%	1	6.6%
21-40	7	29.2%	3	20%
41-60	4	16.7%	5	33.3%
61-80	13	54.2%	6	40%
Total	24	100.0%	15	100.0%

269(89.6%) patients took antibiotics at some point or the other after being diagnosed with cholelithiasis while 31(10.4%) patients had no history of antibiotic intake after diagnosis. Out of 269 patients who took antibiotics, 34(12.7%) had a positive Bile Culture, and out of 31 patients who did not take antibiotics, 5(16.1%) had a positive Bile Culture. (p-value = 0.575 i.e., > 5, which was not significant).

Table 2: Prior antibiotic intake vs Bile Culture Growth

	Prior history of antibiotic intake after being diagnosed with cholelithiasis		Total	
	No	Yes		
Bile Culture Growth	No	26(83.9%)	235(87.3%)	261(87.0%)
Yes	5 (16.1%)	34 (12.7%)	39(13%)	
Total	31 (100%)	269 (100%)	300 (100%)	

22(56.4%) patients who had Type 2 Diabetes mellitus, 16(41.0%) patients who had Hypertension, 2(5.1%) patients who had COPD, 1(2.6%) patient who had CAD, 9(23.1%) patients who had Hypothyroidism and 1(2.6%) patient who had Prostatomegaly, had Bile Culture positive.

Among the microorganisms that were cultured from the positive samples, 3(7.7%) were Bacteroides, 23(59.0%) were E. coli, 10(25.6%) were Klebsiella and 3(7.7%) were Salmonella.

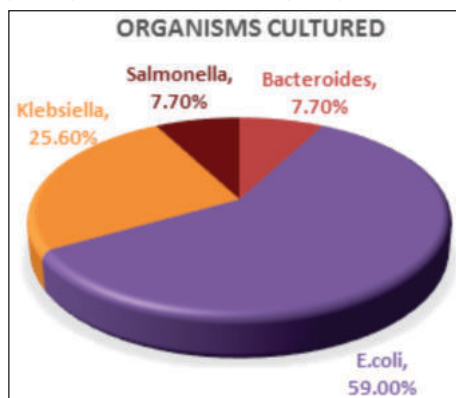


Fig 1: Organisms cultured.

Antibiotic sensitivity of the positive bile cultures showed that, 1(2.6%) was sensitive to Ampicillin, 11(28.2%) were sensitive to Cefuroxime, 8(20.5%) to Ciprofloxacin, 4(10.3%) to Levofloxacin, 5(12.8%) to Meropenem, 5(12.8%) to Metronidazole, 2(5.1%) to Piperacillin-tazobactam and 3(7.7%) was resistant to the antibiotics.

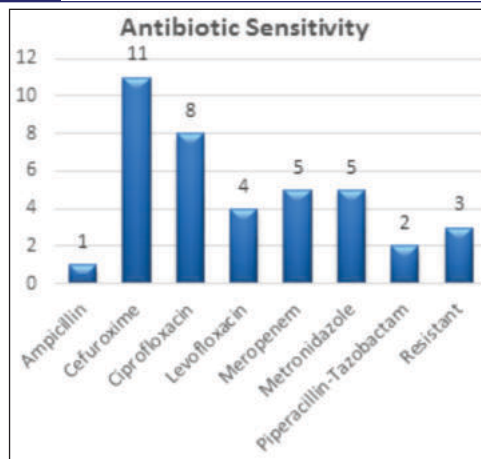


Fig 2: Antibiotic Sensitivity of organisms cultured.

297(99%) patients had undergone Laparoscopic Cholecystectomy, out of which 36(92.3%) patients developed a positive Bile Culture. 3(1.0%) patients who had undergone Laparoscopic converted to Open Cholecystectomy, all 3(7.7%) developed a positive Bile Culture.

Among the Bile Culture positive patients, 6(15.4%) patients had Mild Adhesions at Calot's triangle, 6(15.4%) had Dense Adhesions at Calot's triangle, 3(7.7%) had Mirizzi Syndrome, 4(10.3%) had Mucocoele of gallbladder and in 20(51.3%) patients, intra-operative findings were unremarkable.

DISCUSSION

In our study, majority of patients suffering from Chronic Calculous Cholecystitis were Females(72.3%) and majority belonged to the 41-60 years age group(42.3%). This was similar to Parekh PM et al⁸ study, where maximum number of patients undergoing cholecystectomy were in the 41-50 years age group, with Females being the majority.

Bile Culture was positive in 39(13%) patients, of which majority were Females(61.5%) and majority were in the 61-80 age group for both. In Parekh PM⁸ study, 24.3% patients had a positive Bile Culture, Females being the predominant, belonging to 19-30 years age group. In a study by van Leeuwen et al¹⁰, positive Bile Culture was present in 16.4% patients.

Morgan CG¹¹ concluded that prophylactic antibiotics prevent infections even though they do not sterilize bile. Out of 300 patients, the one who had a prior history of antibiotic intake after being diagnosed with Cholelithiasis(89.7%), Bile culture was positive in 12.7% cases, while in those who had no history of antibiotic intake (10.3%), Bile culture was positive in 16.1%.

Majority of patients who were tested positive for microorganisms in Bile aspirates were suffering from Type 2 Diabetes mellitus(56.4%), followed by Hypertension(41%).

Gram negative anaerobes were the most common organism cultured in Bile. In our study, it was E.coli(59%), followed by Klebsiella (25.6%). In Parekh PM⁸ study also, the most common organism isolated was E.coli(15.8%) followed by Pseudomonas(3.85%) and Klebsiella(2.56%). In Suri et al⁹ study also, E.coli (53.84%) was one of the most common isolated bacteria followed by Pseudomonas aeruginosa (26.92%) and Staphylococcus aureus (19.23%). In van Leeuwen¹⁰ study also, E.coli was the most common organism(36%).

Majority of the microorganisms isolated from the Bile aspirates were sensitive to Cefuroxime (28.2%), followed by Ciprofloxacin (20.5%). Metronidazole was found sensitive in

12.8% cases whereas, Levofloxacin, Meropenem and Piperacillin-Tazobactam were sensitive in 10.3%, 12.8 and 5.1% cases. 3 cases (7.7%) were found to contain microorganisms resistant to commonly used antibiotics. In Parekh PM⁸ study, most of the organism showed sensitivity against Cefoperazone-sulbactam, Piperacillin-Tazobactam, Levofloxacin and Meropenem, while one patient had a multidrug resistant E.coli strain. In Suri et al⁹ study, organisms were sensitive to Cefuroxime, Cefoperazone and Cefepime.

In our study, 3(1.0%) patients who had undergone Laparoscopic converted to Open Cholecystectomy, all 3(7.7%) developed a positive Bile Culture. In Parekh PM et al⁸ study, from 19 patients in which organism were isolated, 14 patients underwent laparoscopic cholecystectomy and in 5 patients, laparoscopic cholecystectomy needed to be converted to open cholecystectomy.

CONCLUSION

In majority of cases, bile was sterile. Only 13% patients had a positive Bile Culture, with enterobacteria being the most frequent isolate, majority sensitive to Cephalosporin. Though our series is probably too small to draw emphatic conclusions, still it seems that infection plays an important role in biliary tract disease.

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