

THE STUDY OF INCIDENCE OF BILIARY INFECTION IN GALL STONE DISEASE IN A HOSPITAL

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ABSTRACT

BACKGROUND

Biliary calculus disease is one of the most common disorders affecting the gastrointestinal tract and is an important cause of morbidity. In about half of the cases, bacteria can be cultured from the gallbladder bile. 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 today.¹ Evidence in favour of infection includes isolation of *E. coli*, bacterium typhosum, streptococcus from the gallbladder bile and from the centre of the gallstones.² Knowing the bacteriological aetiology will help in prevention as well as better treatment of gallstone disease in the future.

MATERIALS AND METHODS

This is descriptive study which includes cases of cholelithiasis, age between 21 to 80 years, who underwent cholecystectomy from June 2014 to June 2016 at CPR Hospital attached to RSCM Government Medical College Kolhapur. Cases of acute cholecystitis, acute acalculous cholecystitis, empyema, mucocele were excluded from the study. The objectives of this study were 1. to determine frequency of infection in cholelithiasis, 2. to find common infecting organisms and 3. To determine their antibiotic sensitivity. Sample size was calculated as 100 using Sample Size formula $Sample\ size = Z^2 * (p) * (1-p) / c^2$. Relevant laboratory investigations like CBC, BSL, LFT with liver enzymes were done. Relevant radiographic investigations like USG were done. Patients were operated either by open or laparoscopic technique. Antimicrobial prophylaxis with 1 gm Ceftriaxone preoperatively and patients who had diabetes mellitus or spillage of bile or stone into the peritoneal cavity continued receiving 1 gm Ceftriaxone every 12 hours postoperatively. Bile and gallstones collected by sterile technique were then put into sterile bottles and transferred to laboratory where they were crushed and cultured to identify organisms and their sensitivity. Statistical analysis was done by Chi square test and Fisher's exact tests.

RESULTS

All patients had cholelithiasis and chronic cholecystitis. Bacteria were isolated in 36 samples (36%). Frequency of isolated bacteria in females and males had no significant difference ($P > 0.05$). Monomicrobial and polymicrobial infection were observed in 34 (94%) and 2 (6%) respectively. *E. coli* was the most common isolate (12; 33%). Enterobacter was the second one (8; 22%). The antibiotics to which organisms were sensitive were Cefepime, Ceftriaxone, Imipenem, Amikacin, Gentamycin and Ciprofloxacin.

CONCLUSION

1. Most common organism detected in bile culture was *E. Coli* followed by enterococcus and anaerobic bacteria.
2. Most effective antibiotic in bile and stone culture positive cases were Cefepime, Ceftriaxone, Imipenem, Amikacin, Gentamycin and Ciprofloxacin.
3. We recommend starting antibiotics selectively, if supported by the clinical conditions or co-morbidities like diabetes or immune-compromised states and culture positive in SSI wound infections according to its culture and sensitivity, in case of cholecystitis and cholelithiasis.

KEY WORDS

Biliary Infection, Gallstone, Antibiotic Susceptibility.

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BACKGROUND

Biliary calculus disease is one of the most common disorders affecting the gastrointestinal tract and is an important cause of morbidity. There has been marked rise in the incidence of gall stone disease in the west during the past century. In the UK, USA and Australia, the prevalence rates varies from 15-25%. In India, it is more common in the North India than in the South India. Similarly the incidence in Eastern India is higher than in Western India.

Incidence of gallstones increases with the age. It is more common in female than male (M: F = 1:4) and about 50% of patients are asymptomatic.¹ The biliary tract is usually sterile; however, if cholelithiasis occurs, different microbes might be identified in and/or cultured from the bile or gallbladder wall. Microscopic examinations indicated that 20-50% of the patients with chronic cholecystitis have a positive bile culture. 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.²

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 today.¹

Evidence in favour of infection includes isolation of *E. coli*, bacterium typhosum, streptococcus from the gallbladder bile and from the centre of the gallstones.² Slow growing actinomyces also have been recovered from the bile. These organisms reaches the gallbladder via blood stream, from infective focus elsewhere in the body and by lymphatics also.³ Brown pigment gallstones occurs as a result of infection. Bacteria are found within the calcium bilirubinate and protein matrix of brown pigment gallstones.⁴ Gallstones may be due to liver disease, gallbladder disease or due to haemolytic anaemia.⁵

As prophylaxis, a number of antibiotics could be prescribed to prevent potential post-cholecystectomy infections. The rate of post-operative wound infection after elective cholecystectomy varies from 7%-20%. Thus, understanding the most common organisms causing them and their antibacterial susceptibility pattern would be useful in prevention of these infections as well as, We can give prophylactic antibiotics in asymptomatic gall stone disease that would prevent acute complications of gall stone disease.

MATERIALS AND METHODS

It is a "Descriptive study", conducted on Patients in age group between 21 to 80 years, admitted to Chhatrapati Pramila Raje Hospital attached to RSCM GMC Kolhapur with the diagnosis of Gallstone disease by ultrasonography were taken for this study and Acute cholecystitis, Acute acalculous cholecystitis, Empyema gall bladder, Mucocele of the gall bladder, Gallstones with multiple common bile duct stones (Multiple CBD and intrahepatic stones) were excluded from study which was conducted from April 2014 to April 2016.

Sample size was calculated, Based on previous studies incidence of biliary infection varies from 20% to 40%, hence, Considering confidence interval of 10 and Confidence level 95%; in a population of Kolhapur region which is around 6 lakh the sample size was calculated as 100 using Sample Size formula-

$$\text{Sample Size} = Z^2 * (p) * (1-p) / c^2$$

Where: Z = Z value (e.g. 1.96 for 95% confidence level)

p = percentage picking a choice, expressed as decimal (.5 used for sample size needed)

c = confidence interval, expressed as decimal.

All our patients received antimicrobial prophylaxis with 1 gm Ceftriaxone preoperatively and patients who had diabetes mellitus or spillage of bile or stone into the peritoneal cavity continued receiving 1 gm Ceftriaxone every 12 hours postoperatively.

Bile is aspirated from the gallbladder of the patient who underwent open cholecystectomy using a sterile syringe (10ml). In case of laparoscopic cholecystectomy, bile is collected from excised gall bladder. The sample is put in sterile bottle and transferred to laboratory immediately.

Gallstones were removed from the gallbladder after the specimen is taken out by open cholecystectomy or laparoscopic cholecystectomy and were transferred to Microbiology Laboratory of Medical Faculty in sterile container where they were crushed and incubated at 37°C for 48 hours to identify organisms and their sensitivity. The samples were cultured in thioglycolate medium, Blood agar and MacConkey's agar to detect aerobic and other bacteria. Differential tests such as IMVIC (Indole, Methyl Red, Voges presquare, and Citrate), motility, urease, and lysine decarboxylase were performed after staining and microscopic observation.

The agar was prepared and sterilised according to the manufacturer's instructions. It is read after 48 hours for growth of organism. After the bacteria were isolated, we performed antibiotic sensitivity tests by the isolates carried out using Kirby-Bauer method. The applied antibiotic disks were Penicillin, Ampicillin, Amikacin, Gentamicin, Cefotaxime, Cefepime, Erythromycin, Clindamycin, Vancomycin, Tetracycline, Novobiocin, Ceftriaxone, Imipenem, Cotrimoxazole and ciprofloxacin.

The results were reported as susceptible, resistant and intermediate based on the diameter of the clear zone around disks with reference to the antibiotic standard table.

Statistical analysis of parameters was done, Data was expressed as percentage and mean $\pm$ standard deviation. Chi square test and fisher's exact tests were used to assess the significance of frequency distribution of data. SPSS VS 16 (IBM CORP) and Microsoft excel 2007 (Microsoft Corp.) were used to perform the statistical analysis. $P < 0.05$ was considered to be statistically significant.

Figure 1



Figure 2



Figure 3



Figure 1, 2, 3. Transportation of Gallstones to the Laboratory

Statistics

Organism	Bile Culture Report	Cholesterol Stone	Pigment Stone	%
Anaerobic bacteria	3	2	2	20%
Coagulase -ve Staph	1	1	1	8%
E. Coli	4	5	3	34%
Enterobacter	4	4	0	23%
Klebsiella	1	1	0	5%
Proteus	1	0	0	3%
Salmonella typhi	1	2	0	8%
Total	15	21		100%

Table 1. Bile and Stone Culture Findings in Study Subjects

- Stone culture findings were compared between types of stones. The Frequency of culture positivity was found to be significantly higher in cholesterol stones (15(34.9%)) compared to that in pigment stones (6 (11.5 %)) and in Mixed stones (0 (0%)) ($p=0.01$) on assessment by Fisher's exact test.
- Bile culture findings were compared between types of surgeries. The Frequency of culture positivity was found to be higher in open cholecystectomy (7(26.9%)) compared to that in laparoscopy (9 (12.1 %)) on assessment by Chi square test but the difference failed to reach the statistical significance ($p=0.07$).

Ethical approval for the study has been taken from the Ethics Committee of RSCM GMC, Kolhapur.

RESULTS

In this study, 100 patients hospitalised due to cholelithiasis were included. All of the patients had cholelithiasis and chronic cholecystitis. The study population included 71 (71%) female and 29 (29%) male with a mean age of 43 ± 16.8 years (range; 21 and 74 yrs.) (Table 3).

Among the total 100 bile and gall stone samples, bacteria were isolated in 36 samples (36%). Frequency of isolated bacteria in females and males had no significant difference ($P>0.05$).

Monomicrobial and polymicrobial infection were observed in 34 (94%) and 2 (6%) respectively. E. coli was the most common isolate (12; 34%). Enterobacter was the second one (8; 23%) followed by Anaerobic bacteria (7; 20%), Salmonella (3;8%), Coagulase-negative Staphylococcus (3; 8%), Klebsiella pneumoniae (2; 5%) and Proteus (1; 3%).

Antibiotic susceptibility tests were performed for organisms that were isolated from the bile samples and the following data were obtained. The most susceptible antibiotic for microorganisms overall was Cefepime, Ceftriaxone, Imipenem, Amikacin, Gentamycin and Ciprofloxacin.

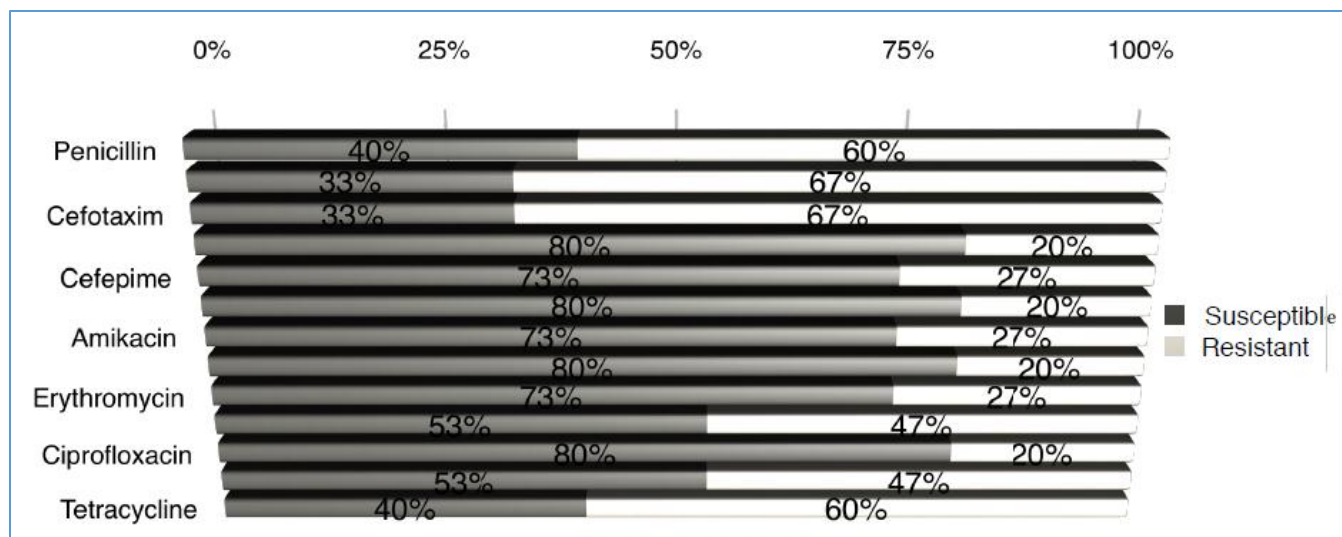


Table 2. Antibiotic Susceptibility in Bile Culture Positive Subjects

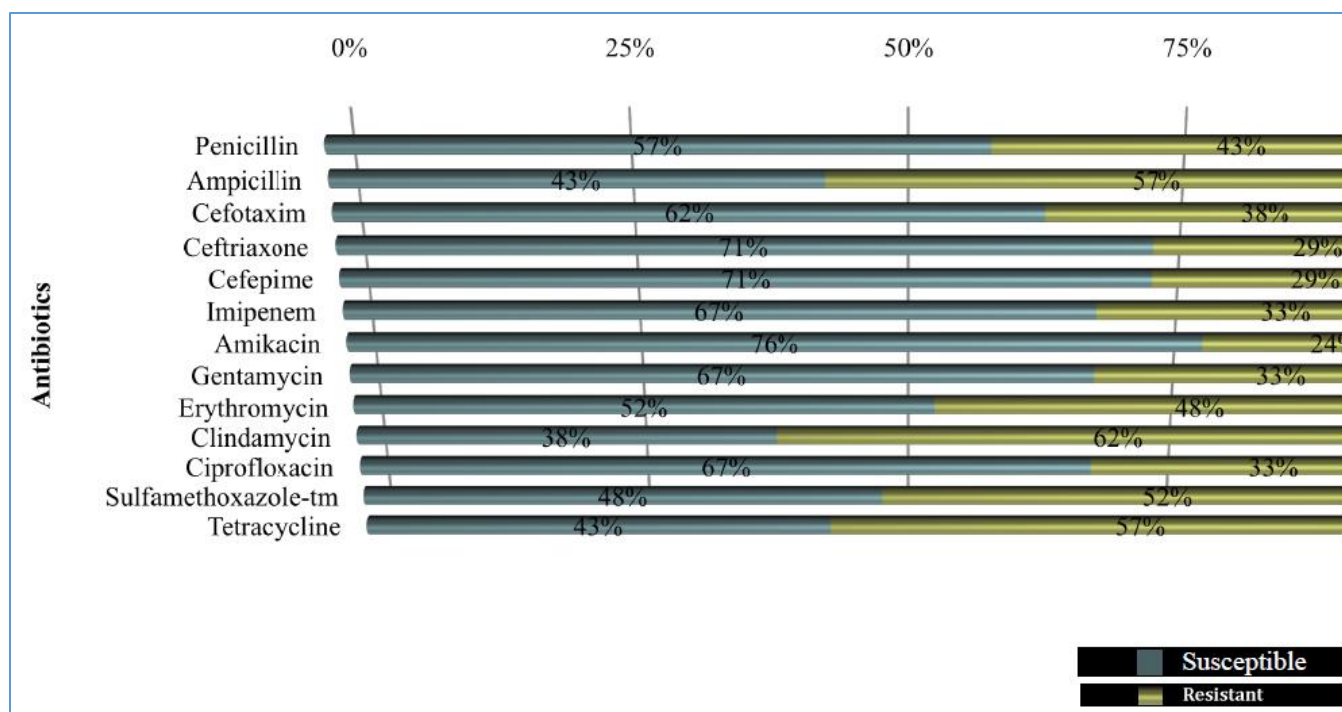


Table 3. Antibiotic Susceptibility in Biliary Stone Culture Positive Samples

DISCUSSION

Calculus cholecystitis with its complications dominates the diseases of the biliary tract. There is an inherent difficulty in identifying patients having infected gall bladders who may have the risk of wound infection or gram negative septicaemia after cholecystectomy. This study is conducted to ascertain the bacteriological patterns and antibiotic sensitivities of bile in calculus cholecystitis patients presenting at a tertiary care hospital and formulate guidelines for prophylactic antibiotic therapy in patients with asymptomatic gall stone disease to prevent complications.

In the Department of Surgery, RSCM GMC Kolhapur, we made our sincere attempt to explore the association between bile infection in gall stone disease. We also tried to explore infection of bile and its association with different type of gall stone formation. (Cholesterol, pigment and mixed type of

gallstones). We have compared our study with other studies done previously for the results of our study.

I. Bacteriology of Bile and Gall Stone

From the total 100 bile and stone samples, bacteria were isolated in 36 samples (36%), of which 15 samples of bile and 21 gall stones were positive for growth of organisms. In the majority of publications 25-35% patients undergoing biliary surgery were found to harbour bacteria in the bile.⁶

In his study by Mohammad Moazeni-Bistgani and Reza Imani on bile bacteria of Patients with Cholelithiasis and Their Antibigram, in Shahrekord University of Medical Sciences, Shahrekord, Iran they collected a total of 132 samples from the patients were tested for bacterial strains using the appropriate methods for testing them. Fifty of 132 (37.87%) studied patients were positive for bacteria in bile culture.⁷

Anaerobic bacteria were detected in 5 (14%),

monomicrobial infection in 34 (94%), and polymicrobial infection in 2 (6%) patients.

In a study by Ballal et al. in India, bile cultures for aerobic and anaerobic bacteria were carried out on 125 samples from patients with chronic cholecystitis with cholelithiasis; 71 (56.8%) aerobic and 17 (13.6%) anaerobic bacteria were detected. Among the mixed flora, 1 had only aerobes and the remaining had both aerobes and anaerobes.⁸

The effect of anaerobic bacteria on bile pigment stone has been reported widely, according to which the anaerobes such as *B. fragilis* and *B. fusiformis* may produce an *E. coli* substance called β -Lactamase which resolves bilirubin. The bilirubin when integrated with calcium ion forms calcium bilirubinate.^{9,10}

The difference in Aerobic and anaerobic positive culture between our study and others could be attributed to the method of antibiotic therapy;

We ordered antibiotic prophylaxis routinely while in Ballal et al. study and Al Harbi et al. study were selectively given.

In our study *E. coli* was the most common isolate (12; 34%). In R.G. Willis and Lawson study it was *E. coli* and in Mohammad Moazeni-Bistgani and Reza Imani series too was *E. coli*. The significance of *E. coli* dominance is also supported by previous reports indicating a potential role for *E. coli*'s glucuronidase enzymatic activity in formation of calcium bilirubinate gall stone.^{8,11}

In our study, *Salmonella typhi* grew in 8% cases, sensitive to Amikacin and Ceftriaxone. The prevalence of *Salmonella typhi* in bile of cholelithiasis patients varied widely from 1% to 34% perhaps due to typhoid fever which is, similar to some parts of our country, endemic in some regions.^{12,13} Analysis of our patients showed that *Salmonella typhi* was more common in females compared to males.

Their high incidences in females have been attributed to hormonal effects related to menstrual cycle and pregnancy.¹⁴

II. Antibiotic Susceptibility

In our study Organisms were most susceptible to Cefepime (72%), Ceftriaxone (76%), Imipenem (73%), Amikacin (75%), Gentamycin (74%) and Ciprofloxacin (73%).

Ballal et al. have shown anaerobes were sensitive to Cefotaxime, Metronidazole, Chloramphenicol, Cefazolin, and Tetracycline, and aerobes isolated to Ampicillin, Chloramphenicol, Streptomycin, Tetracycline, Gentamicin, and second generation Fluoroquinolones such as Ciprofloxacin and Norfloxacin.

Given our and others' studies anaerobes are rare in the human biliary system; therefore, if antibiotic therapy is considered, aerobic coverage should be satisfactory.

In addition because the most susceptible antibiotic for microorganisms on the whole was Ceftriaxone and Amikacin these antibiotics can be started selectively in patients with immunocompromised or diabetic patients as a prophylaxis or in the post-operative period. All our observations in the present study are compared with other studies.

Summary

In our present study, 100 cholelithiasis cases were observed-

1. Most common organism detected in bile culture is *E. coli* followed by enterococcus and anaerobic bacteria.
2. The Frequency of culture positivity was found is significantly higher in cholesterol stones compared to that in pigment stones.
3. Most effective antibiotic in bile and stone culture positive cases are Cefepime, Ceftriaxone, Imipenem, Amikacin, Gentamycin and Ciprofloxacin.

Recommendation

1. Ultrasonography of abdomen is most important investigation for diagnosis of gall bladder stones and advised in all patients have symptoms of gall stone disease.
2. Routine culture of all bile samples is not advised, during surgery.
3. We recommend starting antibiotics selectively, if supported by the clinical conditions or co-morbidities like diabetes or immuno-compromised states and culture positive in SSI wound infection according to its culture and sensitivity, in case of cholecystitis and cholelithiasis.
4. Given our and others studies anaerobes are rare in the human biliary system; therefore, if antibiotic therapy is considered, aerobic coverage should be satisfactory.

CONCLUSION

1. Most common organism detected in bile culture was *E. coli* followed by enterococcus and anaerobic bacteria.
2. The frequency of culture positivity was found to be significantly higher in cholesterol stones compared to pigment stones.
3. Most effective antibiotic in bile and stone culture positive cases are Cefepime, Ceftriaxone, Imipenem, Amikacin, Gentamycin and Ciprofloxacin.

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