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A prospective observational study to study and correlate the clinical and microbiological profile of bile cultures in patients with symptomatic cholelithiasis

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ABSTRACT

Background: Biliary infection has been reported in a significant proportion of patients developing gallstones. Many studies have found biliary microflora in 20% to 46% patients with a post-operative infection rate of 7% to 20% in those who undergo cholecystectomy for symptomatic gallstone disease. Various antibiotics are also used empirically as prophylaxis against post-operative infection. The study was conducted in order to determine the bacteriology and to test its sensitivity to commonly used antibiotics of aspirated bile samples taken during laparoscopic cholecystectomy, and to correlate it with the clinical profile recorded in patients with symptomatic cholelithiasis.

Methods: A total of 266 patients who underwent laparoscopic cholecystectomy were included in the study over a two year period. Intra operatively, bile was aspirated from gall bladder and sent for culture and antibiotic sensitivity testing.

Results: 14.66% (39 out of 266) patients had positive growth with *Escherichia coli* most commonly isolated in 9.77% (26/266) patients, followed by the *Klebsiella* species in 4.89% (13 out of 266) patients. All the cultures were sensitive to amikacin and meropenem, 97.44% to imipenem, and only 43.39% were sensitive to ampicillin. A statistically significant correlation was observed between a positive bile culture with the duration of symptoms (p=0.01874) and gall bladder thickness (p<0.0001). No correlation was seen between bile culture and history of acute cholecystitis, number or size of calculi.

Conclusions: The results of this study can help develop local guidelines and recommendations based on Indian data to ensure the rational use of prophylactic antibiotics in patients with symptomatic cholelithiasis.

Keywords: Bile culture, Biliary microflora, Cholelithiasis, Prophylactic antibiotic

INTRODUCTION

Cholelithiasis is a disease of the hepatobiliary system and impaired metabolism of cholesterol; bile acids and bilirubin are associated with gallstone formation. In India, the prevalence of cholelithiasis ranges between 2% to 29% and is showing increasing trends in recent years. Biliary infection has been reported in a significant

proportion of patients developing gallstones; the infection can be caused by gram-negative, gram-positive or anaerobic organisms. Studies have found biliary microflora in 20% to 46% patients with a post-operative infection rate of 7% to 20% in those who undergo cholecystectomy for symptomatic gallstone disease.² Commonly cultured organisms include *Escherichia coli, Enterococci. Klebsiella. Pseudomonas* and some rare

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organisms. Various antibiotics are used empirically as prophylaxis against post-operative infection.³ There is a wide variation in the reported incidence of positive bile cultures and antibiotic sensitivity in the literature. This study was thus planned in order to determine the bacteriology and to test its sensitivity to commonly used antibiotics of aspirated bile samples taken during laparoscopic cholecystectomy; and to correlate it with the clinical profile recorded in patients with symptomatic cholelithiasis.

METHODS

This prospective observational study was conducted at Armed Forces Medical College and Command Hospital Complex, Pune after obtaining permission from the Institutional Ethics Committee. The study population were all patients (over 18 years of age) who underwent laparoscopic cholecystectomy for symptomatic chole-lithiasis over two years from Jun 2017 to May 2018. A total of 266 patients were included in the study. After the diagnosis was confirmed using ultrasonography, the patients underwent laparoscopic cholecystectomy. They were given one dose of the prophylactic antibiotic (Inj Cefotaxime 1gm IV) at induction of anaesthesia and two doses of the antibiotic post operatively. Intra operatively, the bile was aspirated from gall bladder and sent for culture and antibiotic sensitivity testing.

Baseline study participant characteristics were described using descriptive statistics. Categorical data were analysed using Chi-square test. Parametric correlation analysis were done using Pearson correlation test while non-parametric correlation analysis were done using Spearman correlation test. The GraphPad Software and MS-Excel were used for data entry and analysis.

RESULTS

The mean age of our study patients was 41.94 (SD 12.04) years. The youngest patient was 19 years old whereas the eldest patient was 76 years old. There was a female predominance with 177 females out of 266 patients (66.54%) which was statistically significant (p<0.0001 calculated using sign and binomial test).

The gall bladder was found to be non-distended on ultrasonography in 66.92% (178 out of 266) patients and contracted in 2.26% (6 out of 266) patients. The gall bladder wall was thickened in 15.41% (41 out of 266) patients, while it was normal in the rest 79.32% (211 out of 266). 14 (5.26%) patients had gall bladder sludge and 4 (1.50%) had gall bladder polyps. The mean duration of symptoms among our study patients was 8.73 ± 11.78 months with the duration of symptoms ranging from 1 month to 120 months. Only 2.63% (7 out of 266) patients had a history of acute cholecystitis, while no such history was reported by the remaining 97.37%. An equal distribution of patients with single and multiple calculi was also observed, i.e. 133 out of 266 (50%) each. The

mean size of largest calculi was 12.12±3.84 mm with the size ranging from 4 mm to 21 mm. In present study, most patients (90.23%; 240 out of 266) did not have any comorbidity while 17 (6.39%) patients had diabetes mellitus, 8 patients had hypertension, and 3 (1.13%) had gall stone pancreatitis.

On culture of the aspirate, no bacterial growth was seen in 85.34% (227 out of 266) patients. However, in 9.77% (26 out of 266) patients, *E. coli* were isolated and in 13 (4.89%), *Klebsiella* was grown. The demographic profile and cultures are summarised in Table 1.

Of the 39 patients in whom organisms were isolated, bile culture antibiotic sensitivity showed 43.39% (17) sensitive to ampicillin, all 39 (100%) sensitive to amikacin and meropenem, 84.61% (33) to ceftriaxone, 92.31% (36) to cefotaxime, 97.44% (38) to imipenem, and 94.87% (37) were sensitive to gentamicin (Table 2).

Table 1: Patient demographics and bile culture isolates.

Factors	N (%)			
Mean age (years)	41.94 (SD 12.04)			
Gender				
Female	177 (66.54)			
Male	89 (33.46)			
Ultrasonography: distension				
Non distended	178 (66.92)			
Partially distended	7 (2.63)			
Distended	30 (11.28)			
Contracted	6 (2.26)			
Ultrasonography: wall thickness/ contents				
Normal	211 (79.32)			
Thickened	41 (15.41)			
Sludge	14 (5.26)			
Polyps	4 (1.50)			
Duration of symptoms				
Duration (months)	8.73 (SD 11.78)			
History (acute cholecystitis)				
Yes	7 (2.63)			
No	259 (97.37)			
Number and size of Calculi				
Single	133 (50)			
Size of largest calculus (mm)	12.08 (SD 3.84)			
Organism isolated				
No growth	227 (85.34)			
E. coli	26 (9.77)			
Klebsiella	13 (4.89)			

The Spearman's correlation coefficient (r) was used to assess the correlation of bile culture with various parameters. A statistically significant correlation was observed between a positive bile culture, i.e. the presence of organisms in bile culture with the duration of symptoms (p=0.01874) and gall bladder thickness (p<0.0001). Whereas no correlation was seen between

bile culture and history of acute cholecystitis (p=0.26811), number of calculi (p=0.38802) and the size of the largest calculi (p=0.89212) (Table 3).

Table 2: Bile culture antibiotic sensitivity.

Culture	Sensitivity		Resistance	
findings	N	%	N	%
Ampicillin	17	43.49	22	56.41
Amikacin	39	100	0	0
Ceftriaxone	33	84.61	6	15.38
Cefotaxime	36	92.31	3	7.69
Imipenem	38	97.44	1	2.56
Meropenem	39	100	0	0
Gentamicin	37	94.87	2	5.13

Table 3: Correlation of bile culture with various parameters.

Parameter	r	P value
Duration of symptoms	0.14406	0.01874
History of acute cholecystitis	-0.06814	0.26811
Number of calculi	0.05314	0.38802
Size of largest calculi	-0.00836	0.89212
Gall bladder thickness	0.28169	< 0.0001

Correlated using Spearaman's correlation coefficient (r); Where r=1 means a perfect positive correlation and r=-1 means a perfect negative correlation

DISCUSSION

Biliary infection has been reported in a significant proportion of patients developing gallstones; the infection can be caused by gram-negative, gram-positive or anaerobic organisms. Around 94% of biliary infections are caused by aerobic organisms, while anaerobic organisms account for the rest. Gram negative enteric aerobes such as *E. coli, Klebsiella* species and *Proteus* species, are more common while *Pseudomonas aeruginosa, Bacteroides fragilis and Enterococcus faecalis* are less commonly cultured. The enzymes secreted by the bacteria can alter bile composition which can in turn lead to precipitation of bile salts. It remains unknown whether the bacteria colonisation in bile results in gallstone formation or vice-versa, but bacterial biofilm cover most gallstones even if the bile remains bacteria-free.

If the bile duct gets obstructed, then bacteria can proliferate within the stagnant bile. Bacteria can then enter the circulation leading to a systemic infection. Bacteria can be isolated from the bile or gallbladder in about 30% of cholelithiasis patients. Few studies have found that certain bile bacteria like *Helicobacter pylori* can induce cholesterol gallstone formation.⁷

Bacterial presence in bile has also been reported to be associated with an increased risk of adverse postoperative outcomes post laparoscopic cholecystectomy.⁸ This study

was thus planned in order to determine the bacteriology of aspirated bile samples and to test its sensitivity to commonly used antibiotics and to correlate it with clinical presentation in patients with symptomatic cholelithiasis.

In the present study, on culture and sensitivity test, 14.66% (39 out of 266) patients had positive growth while no bacterial growth was seen in 85.34% (227) patients with findings similar to a UK study with culture positivity in 15.6% of patients.⁹ In cultures *E. coli* was more commonly isolated in 9.77% (26) patients, followed by the *Klebsiella* species in 4.89%. Of the 39 patients in whom organisms were isolated, bile culture findings were as follows: less than half (43.39%) were sensitive to ampicillin, 100% (39 out of 39) were sensitive to amikacin and meropenem, 84.61% (33 out of 39) to ceftriaxone, 92.31% (36) sensitive to cefotaxime. Imipenem and gentamicin also showed a high sensitivity of 97.44% and 94.87% respectively.

A cross sectional study by Ahmad et al reported a higher 58.58% of patients with symptomatic cholelithiasis positive for bacterial growth. *E. coli* was the most common bacteria isolated from such patients while other commonly reported bacterial were *Klebseilla* (17.16%), *Salmonella* (12.68%) and *Shigella* (6.34%) and in more than 50% cases all four bacterial species were found to be sensitive to cefuroxime, ceftriaxone, ciprofloxacin and amoxicillin.⁴

However in the study by Ahmad et al, only 23.6% patients showed positive growth on bile culture and they also observed the presence of *E. coli* (40%) in maximum culture isolates. *E. coli* showed good sensitivity to amikacin, cefoperazone plus sulbactam, imipenem (100%) and ceftriaxone (90%) but resistant to ampicillin (80%) as was seen in our study.¹⁰

Table 4: Comparison of bile culture findings among various studies.

Study	E. coli (%)	Klebsiella (%)
Ahmad et al ¹⁰	40	20
Ahmad et al ⁴	25.74	17.16
Bistgani et al ¹¹	26	4
Capoor et al ¹³	29.7	27
Sattar et al ⁵	17	9
Gomes et al ¹²	40	35
Present study	9.77	4.89

Bistgani et al observed positive growth on bile culture in 37.87% patients while 62.13% had no growth, with monomicrobial infection in 94% patients and polymicrobial infection in 6% patients. *E. coli* was the most common isolate (26%), followed by *Enterobacter* (18%), *Salmonella typhi* (14%), *Coagulasenegative Staphylococcus* (12%) while *K. pneumoniae* was grown in only 4% of cultures. ¹¹ In contrast Gomes et al in Sri

Lanka grew *E coli* in 40 % and a high 35% of *Klebsiella* species (Table 4).¹²

Capoor et al reported that 32% bile samples from acute cholecystitis patients showed presence of bacteria compared to 51.4% with gastrointestinal ailments needing biliary drainage and 1.6% of gallbladder carcinoma patients thus showing a higher correlation with positive bile cultures and acute cholecystitis. However, no correlation was seen in our study between positive bile culture and history of acute cholecystitis (p=0.26811), the number (p=0.38802) or the size of calculi (p=0.89212). This may be a reflection of the smaller number of patients with a prior history of acute cholecystitis in our study.

CONCLUSION

Symptomatic cholelithiasis is commonly seen amongst the female gender. Though gallbladder distension and wall thickening was not observed in most of our study patients, a statistically significant correlation was observed between a positive bile culture with the duration of symptoms and gall bladder thickness. There was no statistically significant association with history of cholecystitis, associated comorbidity, type of calculi (whether single or multiple) and the size of calculi though this may change on a study with a larger sample size. E. coli, followed by Klebsiella are the most commonly isolated organisms, all of which were completely sensitive to amikacin and meropenem and most showed highest resistance to ampicillin. The results of this study can help develop local guidelines and recommendations based on Indian data to ensure the rational use of prophylactic antibiotics in patients with symptomatic cholelithiasis.

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