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Bacteriological Study of Bile in Patients Undergoing Cholecystectomy for Chronic Calculous Cholecystitis

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ABSTRACT

Background: Chronic calculous cholecystitis frequently involves bacterial colonization of bile, contributing to recurrent inflammation and postoperative complications; identifying pathogens and antibiotic sensitivity remains clinically important. **Objective:** To determine bacteriological profiles of gallbladder bile and analyze antimicrobial susceptibility patterns among patients undergoing cholecystectomy for chronic calculous cholecystitis. **Methods:** This cross-sectional descriptive study included 100 consecutive patients undergoing cholecystectomy at Rajshahi Medical College Hospital between January and December 2019. Demographic, clinical, and microbiological variables were recorded using a structured data sheet. Bile samples were cultured and tested for antibiotic sensitivity following CLSI guidelines. Data were analyzed using SPSS version 23, employing chi-square and Student's t-tests with statistical significance set at $p<0.05$. **Results:** The mean age was 37.73 ± 5.19 years (range 28–69). Females predominated (62% vs 38%, ratio 1:1.63). Urban residence accounted for 58%, while 71% had normal nutritional status ($p = 0.0001$). Mixed gallstones were most common (62%), followed by cholesterol (30%) and pigment stones (8%). Bile culture was positive in 41% of patients. Among isolates, E. coli (39.02%) was predominant, followed by Pseudomonas aeruginosa (26.83%), Klebsiella (19.51%), and S. epidermidis (9.76%), while S. aureus and Salmonella typhi each accounted for 2.44%. Culture positivity was significantly associated with mixed stones (87.8%, $p = 0.0001$). Antibiotic sensitivity showed highest response to imipenem (100%), followed by cefuroxime (95.12%), gentamicin (90.24%), ciprofloxacin (85.37%), and ceftazidime (80.48%). Ceftriaxone resistance reached 17.08%. **Conclusion:** Most bile cultures remain sterile, yet significant bacterial colonization occurs in chronic calculous cholecystitis. E. coli predominates, and imipenem shows highest efficacy while ceftriaxone resistance raises concern.

Keywords: Cholecystitis, Bile Culture, Gallstones, Antibiotic Sensitivity, Bacteriology.

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INTRODUCTION

Biliary tract disease is a wide spectrum of clinical term which is dominated by cholecystitis and cholelithiasis with their complications [1]. Chronic calculous cholecystitis is the commonest among them [2]. It has more female preponderance than male [3]. The fourth decade people are the mostly sufferer. The prevalence of gall stone disease is very high. In the United

States, 20.5 million people have the condition, i.e., 6.3 million men and 14.2 million women. Twenty percent of people over the age of 65 have gallstones, and one million new cases are diagnosed every year. Several studies carried out in our country have demonstrated that the prevalence of this entity is approximately 14.3% [4]. The current mean prevalence in Europe is 18.5%, with the lowest prevalence being reported from Ireland (5%) and the highest from Sweden (38%) [5]. Among Asian countries, North Indians have the highest reported rates of cholelithiasis, afflicting 64.1% of women and 29.5% of men. In Bangladesh, there has been no study that determined the prevalence of gall bladder stone still yet. But a previous study revealed that approximately 5% of rural Bangladeshi community usually suffers from gall stone disease [6]. There are different types of gall stones. Primarily they can be divided into three major groups (i) Cholesterol stone, (ii) Pigment stone and (iii) mixed stone. In many cases, bacteria can be cultured from gall bladder 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 within it" is true today [7]. Septic complications reported from stones and concretions lost in the peritoneal cavity following laparoscopic cholecystectomy reflect the infective potential of gallstones. The pathogenesis of gallstone is multifactorial. It varies according to type of gallstone. Different factors have been implicated amongst which infection of bile is also an important factor. Gallstone varies in their composition majority being mixed gallstone which accounts of 80% and cholesterol gallstone contributing of 20%.

Mixed gallstone is frequently associated with cholecystitis. Bile which in normal is sterile is found to be positive in 50% those with gallstone disease. These bacteria reach the gallbladder via bloodstream from infective focus found elsewhere in body. The biliary infection can be caused by any type ranging from aerobic gram positive to gram negative to anaerobic organisms. Aerobic causes 94% of biliary tract infection while anaerobic causes the rest. The most common organism isolated includes E. coli, Klebseilla, and Streptococcus [8]. Prompt administration of the appropriate antibiotics is crucial in the management of biliary tract infection and antimicrobial treatment is commonly administered pre or peri operatively and often inhibits bacterial growth. Moreover, it is suggested that the recovery of bacteria in bile cultures is affected by toxicity of bile salts. Thus, traditional culturing methods of bile might miss a large number of underlying bacterial infections that could lead to acute or chronic cholecystitis [9]. Culture of bile from the gallbladder of patients with uncomplicated cholelithiasis during cholecystectomy has shown principally E. coli. Others include Pseudomonas spp., Enteroccocus faecalis, Streptococcus spp. and Klebsiella spp. [10]. Bile from the gallbladder of patients with uncomplicated cholelithiasis who have different types of gallstones is rarely studied. Also, investigations of type and prophylactic antibiotic sensitivity of microflora in bile from the gall-bladder are rare in patients with different types of gallstones.

MATERIALS AND METHODS

Study Design

This study was conducted as a cross-sectional descriptive investigation. It was carried out in the Department of Surgery at Rajshahi Medical College Hospital (RMCH), Rajshahi, Bangladesh, over a one-year period from January 2019 to December 2019. The target population consisted of patients diagnosed with chronic calculous cholecystitis who were admitted to the surgical wards and scheduled for cholecystectomy. A total of 100 patients were included in the study. Participants were selected using purposive consecutive sampling based on availability and eligibility criteria. Patients of any age and sex presenting with clinical and ultrasonographic evidence of chronic calculous cholecystitis were considered eligible. Patients with complications of chronic cholecystitis, choledocholithiasis, severe systemic illness, prior antibiotic use before admission, or unwillingness to participate were excluded. The main objective of the study was to evaluate bacteriological profiles of bile and determine antibiotic sensitivity patterns among patients undergoing surgical treatment for gallstone disease.

Data Collection

Data were collected using a pre-structured and pre-tested questionnaire designed for this study. Information regarding demographic characteristics, clinical history, nutritional status, and type of gallstone was recorded. Patients underwent detailed clinical evaluation and relevant investigations prior to surgery. During laparoscopic or open cholecystectomy, bile samples were aseptically aspirated from the gallbladder

and transferred into sterile containers. The samples were immediately sent to the Department of Microbiology at Rajshahi Medical College for bacteriological culture and antibiotic sensitivity testing according to standard laboratory protocols and CLSI guidelines. All procedures were performed under supervision of experienced consultant surgeons.

Data Analysis

Collected data were checked for completeness, compiled, and coded prior to analysis. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 23.0 for Windows. Quantitative variables such as age were summarized using mean and standard deviation, while categorical variables were presented as frequency distributions and percentages. Associations between categorical variables were evaluated using the Chi-square test. Continuous variables were compared using Student's t-test when appropriate. A p-value of less than 0.05 was considered statistically significant. The analyzed data were presented in tables, charts, and descriptive summaries to illustrate demographic characteristics, microbiological findings, and antibiotic sensitivity patterns.

Ethical Considerations

Ethical approval for the study was obtained from the Ethical Review Committee of Rajshahi Medical College Hospital prior to initiation of the research (Ethical Approval ID: RMC/ERC/2018/217). Written informed consent was obtained from all participants before inclusion. Confidentiality and anonymity of patient information were strictly maintained throughout the study. Participants were informed about the objectives, procedures, potential benefits, and minimal risks of the study, and they retained the right to withdraw at any stage without affecting their treatment.

RESULTS

Age distribution (n=100)

Table 1: Distribution of Patients According to Age (n=100)

Age group (in years)	Frequency (%)
<30	3
31 – 40	38
41 – 50	29
51 – 60	23
>60	7
Mean age±SD	**37.73±5.19**
Age range	**28 – 69**

Table 1 shows that among 100 patients, the highest 38% were from age group 31-40 years which was subsequently followed by 29% patients in 41-50 years age group. Besides, 23%, 7% and 3% patients belonged to 51-60 years, >60 years and <30 years age group respectively. The mean age of the patients was 37.73±5.19 (age range: 28-69) years.

Sex with area of residence and nutritional status distribution (n=100)

Table 2: Distribution of patients according to sex associated with Area of residence and Nutritional status (n=100)

Sex		Area of Residence		Nutritional Status		
		Urban	*Rural*	*Poor*	*Normal*	*Overweight*
Male	38(38%)	20(52.63%)	18(47.37%)	8(21.05%)	24(63.16%)	6(15.79%)
Female	62(62%)	38(61.29%)	24(38.71%)	9(14.52%)	47(75.81%)	6(9.68%)
Total	100	58	42	17	71	12

P- value is 0.0001[S]

P-value was calculated by chi square test

S: Significant
P-value was significant at <0.05

Table 2 shows that out of 100 patients 62% female and 38% male. Out of 62 female patients 38(61.29%) come from urban & 24(38.71%) from rural. Out of 38 male patients 20(52.63%) come from urban & 18(47.37%) from rural. Out of 62 female patients 9(14.52%), 47(75.81%) and 6(9.68%) had poor, normal and overweight nutritional status respectively. Out of 38 male patients 8(21.05%), 24(63.16%) and 6(15.79%) had poor, normal and overweight nutritional status respectively.
The statistics showed significant difference (P=0.0001).

Type of gallstones (n = 100)

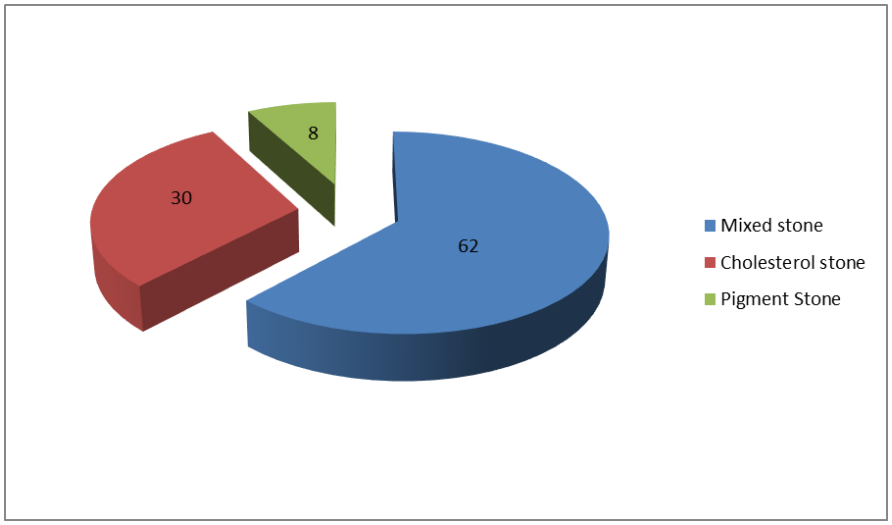

Figure 1: Distribution of patients according to type of gallstones (n = 100)

Figure 1 shows that out of 100 patients 62% had mixed stones, 30% had cholesterol stones, and rest 8% pigment stones.

Bile culture (n = 100)

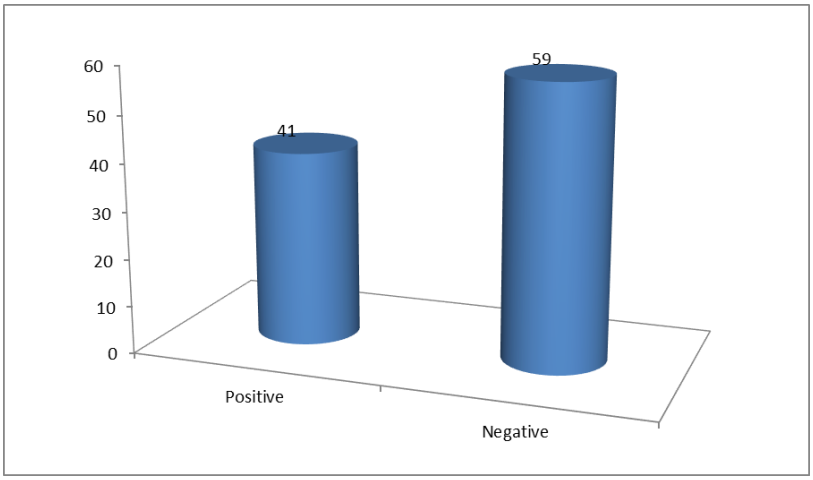

Figure 2: Distribution of Patients According to Bile Culture (n = 10

Figure 2 shows that out of 100 specimen of gall bladder, bile culture revealed 41% had positive culture and 59% had negative culture.

Table 3: Distribution of Patients According to Bile Culture with Associated Gallstone (n=41)

Type of stone	Bile culture positive (n=41)	Bile culture negative (n=59)	Total (=100)	P-value
Cholesterol	4 (9.76%)	26 (44.07%)	30	0.0001[S]
Pigment	1 (2.44%)	7 (11.86%)	8	
Mixed	36 (87.8%)	26 (44.07%)	62	

Bile culture with gall stone variety (n=41)
P-value was calculated by chi square test
S: Significant
P-value was significant at <0.05

Table 3 shows that out of 41 bile culture positive cases, 36(87.8%), 4(9.76%) and 1(2.44%) had mixed, cholesterol and pigment stone respectively. Among 59 bile culture negative cases, 26(44.07%), 26(44.07%), and 7(11.86%) were cholesterol, mixed, and pigment stone respectively. The statistics showed significant difference (P=0.0001).

Table 4: Distribution of Microorganism Isolated from Bile (n= 41)

Bacteria isolated	Frequency (%)
E. coli	16 (39.02%)
Pseudomonas auregenosa	11 (26.83%)
Klebsiella	8 (19.51%)
S. epidermidis	4 (9.76%)
Staphylococcus aureus	1 (2.44%)
Salmonella typhi	1 (2.44%)

Micro-organism isolated (n=41)

Table 4 shows that among 41 culture positive specimen of bile 16(39.02%), 11(26.83%), 8(19.51%) and 4(9.76%) specimen E.coli, Pseudomonas auregenosa, Klebsiella and S. epidermidis were isolated. One (2.44%) each cases staphylococcus aureus and Salmonella typhi was isolated.

Table 5: Distribution of Patients According to Antibiotic Sensitivity (n= 41)

Antibiotic	Sensitivity pattern (n=41)		
	S	MS	R
Imipenem	41 (100%)	-	-
Cefuroxime	39 (95.12%)	-	2 (4.88%)
Gentamicin	37 (90.24%)	3 (7.32%)	1 (2.44%)
Ciprofloxacin	35 (85.37%)	3 (7.32%)	3 (7.31%)
Ceftazidime	33 (80.48%)	4 (9.76%)	4 (9.76%)
Ceftriaxone	31 (75.6%)	3 (7.32%)	7 (17.08%)
Amikacin	22 (53.66%)	-	-

Antibiotic sensitivity pattern (n=41)

*S= Sensitive, MS: Moderately sensitive, R: Resistant

Table 5 shows that imipenem showed the best sensitivity to all sorts of organisms. Besides, cefuroxime, gentamicin, ciprofloxacin, ceftazidime, ceftriaxone and amikacin showed sensitive in 39(95.12%), 37(90.24%), 35(85.37%), 33(80.48%), 31(75.6%) and 22 (53.65%) cases. Ceftriaxone showed alarming resistance in 7 (17.07%) cases.

DISCUSSION

Cholecystitis and Cholelithiasis are prevalent in certain region of the world and quite rare at other places and has been reported in 54% of the adult above 21 years of age [11]. The estimated prevalence of gallstone disease in Indian sub-continent has been reported as 2% to 29%. The average age of these patients in our country is a decade younger than those in western. The maximum number of patient in this study was in the 3rd and 4th decade of life. The mean age of the respondents of this study was 37.73±5.19 (age range: 28-69) years. Golse *et al.*, reported peak age of incidence as 41-50 years [12]. Studies have shown an increase in the prevalence of gallstone with age probably because of decrease in activity of cholesterol reductase and increase in activity of HMG Co-A reductase [13]. Male to female ratio in this study was 1:1.63, in all literature available so far females have been easiest victim of gallstone disease. In fact, female sex hormone and sedentary habits of most women expose them to factors that possibly promote the formation of gallstones. Our study findings of sex distribution was correlated with the studies conducted by Golse *et al* [13]. Among out of 100 patients 58% come from urban area and 42% come from rural area and out of 100 patients 17% were poor, 71% were normal and 12% were overweight in nutritional status. In this study gallstone was classified in cholesterol stones, pigment stones and mixed stones according to their morphological appearance and mixed type of gallstone was found to be predominant 62%, followed by cholesterol stone 30%. and pigment stone 8%. Other studies have also reported similar incidence of type of gallstone. But in a study Wang *et al.*, pigment stones were the most common stones observed [14]. In other study by Jaswal *et al.*, pigment stones were the commonest finding (39%) followed by mixed stones (34%) and cholesterol stones (17%) [15]. In majority of publications, 25% to 50% patients undergoing biliary surgery were found to harbor bacteria in bile. In this study, 41 out of 100 patients were found to be bile culture positive (41%). This finding is similar to the findings of Coccolini *et al.*, [study of Pakistan (36%)] and Million *et al.*, (25%) [16, 17].

In this study incidence of bile culture positivity was more in the cases of mixed type of gallstone 36(87.80%) out of 41, and the most common organism isolated was E.coli 16(39.02%) followed by Pseudomonas auregenosa 11 (26.82%) and Klebseilla 8(19.51%). In other study it is found that mixed stone is frequently associated with cholecystitis. In Bacteria are less often found in the bile of patients with cholesterol gallstones 24% and pigment gallstones 9% to 19%. E.coli was found to be the commonest organism in this study, and it also reported in previous studies. Sudhaharan *et al.*, (46%) study reported E.coli the commonest bacteria isolated [18]. The importance of the predominance of E.coli is seen by the fact that older studies have shown glucoronidase enzymatic activity of E.coli to have role to play in calcium billirubinate gallstone formation. Charlier *et al.*, in their studies concluded that the most susceptible antibiotic for microorganisms was amikacin [19]. Antibiotic sensitivity patterns of isolated organisms were similar, irrespective of the type of stone in the bile. Imipenem and gentamicin may be used as second choice to treat the infection which other drugs can not treat. In our study, we have observed, imipenem was 100% sensitive. Subsequently, cefuroxime (95.12%) and gentamicin (90.24%) revealed a good performance in culture sensitivity. Ceftriaxone showed sensitivity in only 75.6% cases. On the contrary, this drug showed resistance in 17.07% cases. This is a danger turn of antibiotic resistance in our perspective. Our results are comparable to previous study of Geerlings *et al.*, (54%) [20].

CONCLUSION

In case of chronic calculous cholecystitis, majority bile culture are negative. E.coli, Pseudomonas auregenosa, Klebsiella and S. epidermidis are the commonly evident organisms from these bile specimens. Imipenem is highly sensitive to the most of the organisms whereas ceftriaxone shows alarming resistance.

Limitation Of the Study

This was a cross sectional study.
The sample size was too small.
The study period was short.
This study was a single centered, single blinded study.

Recommendation

A case control study is recommended.
A multi-centered, double blinded study is recommended. A large sample size as well as long term study is advocated.

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