Microbiological Profile and Antibiotic Sensitivity Pattern of Bile Culture in Patients Undergoing Laparoscopic Cholecystectomy for Cholelithiasis in Tertiary Care Hospital

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ABSTRACT

Background

Laparoscopic Cholecystectomy is, a frequently performed surgery for symptomatic cholelithiasis. On various studies, in a significant proportion of patients developing gallstones, biliary infection has been noted.

Objective

To assess the bacteriological profile of bile and to determine antibiotic sensitivity pattern for preoperative prophylaxis in laparoscopic cholecystectomy patients.

Method

A prospective observational study was carried out from November 2021 to April 2022 at the Department of Surgery, Shree Birendra Hospital, Chhauni, Kathmandu, Nepal after obtaining ethical approval from the Institutional Review Committee of Nepalese Army Institute of Health Sciences (Reg. No: 478) and informed written consent from each patient. A total of 123 patients undergoing for laparoscopic cholecystectomy were studied. About 5 ml of bile aspirated from gall bladder was collected in a sterile syringe and transported to laboratory for culture and sensitivity. Demographic characteristics, culture outcomes and postoperative parameters were collected using predesigned Performa. Statistical analysis was performed using Statistical Package for the Social Sciences software (SPSS).

Result

Bile culture was positive in 25 (20.33%) patients. The most predominant organisms were Gram negative bacteria. The most commonly isolated organisms were *Escherichia coli*, *Pseudomonas spp* and *Klebsiella spp.* On sensitivity profile, the isolates were most sensitive to antibiotics like: amikacin, gentamicin, clindamycin, meropenem, imipenem, linezolid and piperacillin plus tazobactum with resistance encountered against ampicillin.

Conclusion

Most common organisms isolated from bile culture were Gram negative. Aminoglycoside group of drugs was found to be most sensitive and can be used as first line drug for preoperative prophylaxis in patients undergoing laparoscopic cholecystectomy. It also highlights on the importance of understanding demographical and clinical aspects of patient's profile along with bactibilia.

KEY WORDS

Antibiotics, Bactibilia, Bile, Cholelithiasis, Laparoscopic cholecystectomy

INTRODUCTION

Cholelithiasis is a worldwide hepatobiliary disease which is known as the common reason for laparoscopic intervention. Following laparoscopic cholecystectomy, significant biliary infection has been noted.[1] Studies have found 20% to 46% patients with biliary microflora develop a post-operative infection rate of 7% to 20% in symptomatic gallstone disease.[2,3] *Escherichia coli*, *Enterococci*, *Klebsiella*, and *Pseudomonas* are the most common organisms isolated in bile following laparoscopic cholecystectomy,[2,4] and various prophylactic antibiotics are used to reduce post-operative infections.[5,6]

The variation in incidence of positive bile isolates and antimicrobial sensitivity varies across the literature with limited research on the biliary tract infections and antibiotic susceptibility in Nepal.[3] Hence, this study could be utilized to provide guidelines for prescribing appropriate prophylactic antibiotics in patients undergoing laparoscopic cholecystectomy.

Therefore, the primary aim of this study was to determine the bacteriology and antibiotic sensitivity pattern of bile samples obtained during laparoscopic cholecystectomy in order to evaluate preoperative prophylaxis in patients suffering from symptomatic cholelithiasis.

METHODS

This prospective observational study was conducted from November 2021 to April 2022 at the Department of surgery, Shree Birendra Hospital, Chhauni, Kathmandu, Nepal.

A single institute based purposive sampling technique was used for sampling method. Few studies on similar topics are available in literature. In a study by Yun et al. 25.1% of positive bile culture was found in patients with cholelithiasis who underwent laparoscopic cholecystectomy.[7] We calculated the sample size using Cochran's formula (at 95% Confidence Interval (CI) and 90% power) as follows:

Minimum required sample size (N) = $Z^2pq/L^2 = 73$

Where, Z -value =1.96 at 95% CI, p =25, q =75, and L =10% =0.10

By adding 20% of no response rate, a total of 123 samples were taken for our study.

Once the surgery was performed, the bile sample for culture and sensitivity of individual patient along with HPE of GB was sent. The report was collected during follow-up. In all cases, complications (recognized intra-operatively or within 04 weeks post-operatively) was noted if any. An operative time, duration of hospital stay and surgical site infection (SSI) was noted and data was analyzed subsequently.

A total of 123 patients who met the inclusion criteria were enrolled in the study. About 5 ml of bile was aspirated during laparoscopic cholecystectomy, in a sterile syringe and transported to laboratory for culture and sensitivity. A pre-designed proforma consisting of demographic characteristics (including gender, age), presence of other medical comorbidities, treatment history, complications and outcomes during hospitalization was used as tool for the data collection. Additional clinical outcomes were extracted from patient's medical records based on the clinical evaluation of surgeon. Ethical approval (Reg. No. 478) was taken from the Institutional Review Committee (IRC) of Birendra Sainik Hospital Before commencement of the study.

All patients above 18 years of age with cholelithiasis undergoing laparoscopic cholecystectomy were included in the study and patients undergoing open cholecystectomy, patients with liver disease, malignancy; carcinoma gall bladder, HIV/immune-compromised, antibiotics received patients with in the last seven days prior to surgery and those not willing for study were excluded from the study.

Statistical analysis was performed using Statistical Package for the Social Sciences software (SPSS) (version 21). The descriptive statistics were presented as means, standard deviations, and frequencies. Independent t-test and Pearson's Chi-square test were applied to determine the association between demographic parameters with culture outcomes and postoperative parameters with culture outcomes respectively. A p-value of less than 0.05 was considered statistically significant.

RESULTS

In this study, patients enrolled for laparoscopic cholecystectomy for cholelithiasis in a time frame of 6 months from November 2021 to April 2022 were above 18 years of age, out of which maximum number of patients 38 (30.8%) were of 50-60 age group, remaining 31 (25.2%) of 40-50 age group, 24 (19.5%) of 30-40, 26 (21.1%) were above 60 and 4 (3.2%) were less than 30 years of age. The study population had a majority of women, specifically 94 (76.4%) female patients were present in total (Table 1) and 80% of patients had no history of any known co-morbidities (Fig. 1).

Table 1. Socio-demographic profile of patients (n=123)

Parameters	Frequency*
Gender	
Male	29(23.5)
Female	94(76.4)
Age (years)	
< 30	4(3.2)
30-40	24(19.5)
40-50	31(25.2)
50-60	38(30.8)
> 60	26(21.1)

*Parenthesis indicates percentage

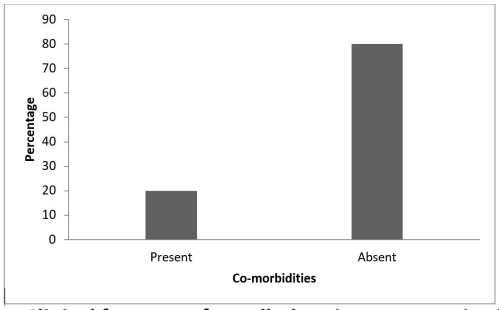

Figure 1. Clinical features of enrolled patients categorized by co-morbidities.

On assessment of intra-operative clinical findings, omental adhesion was the most commonly encountered one with 36 patients (29.2%) followed by bowel adhesion in 17 (13.8%), frozen Calot's triangle in 14 (11.3%), inflamed gall bladder wall in 10 (8.1%), mucocele in 3 (2.4%) and pyocele in 2 (1.6%) patients (Table 2).

Table 2. Assessment of intra-operative clinical findings of enrolled patients (n=123)

Intra-operative findings	Frequency*
Bowel Adhesion	
Yes	17(13.8)
No	106(86.1)
Omental Adhesion	
Yes	36(29.2)
No	87(70.7)
Calot's Triangle Frozen	
Yes	14(11.3)
No	109(88.6)
GB wall Inflammation	
Yes	10(8.1)
No	113(91.8)
Mucocele	
Yes	3(2.4)
No	120(97.5)
Pyocele	
Yes	2(1.6)
No	121(97.3)

* Parenthesis indicates percentage

On analysis of complications, 28 (22.76%) patients suffered from intra-operative complications like injury to bile duct and cystic artery, bile spillage, GB perforation with stone spillage, injury to adjacent organs and conversion to open surgery. None of them had any post-operative complications (Table 3).

Demonstrating frequency of positive bile culture outcome in relation to demographic and intra-operative parameters, among 25 (20.33%) patients with culture positive samples, 5 were male while 20 were female patients. Out of 25 patients having co-morbidities, 8 (32%) had positive culture. Similarly, 9 patients with omental adhesion, 4 with bowel adhesion, 5 with frozen Calot's anatomy, 3 with inflamed GB wall, 1 with pyocele and 2 patients with bile spillage had positive culture results (Table 4).

Table 3. Intra-operative and post- operative complications in patients following laparoscopic cholecystectomy (n=123)

Parameters	Frequency*
Intraoperative	
Bile Duct Injury	1(0.8)
Cystic Artery Injury	1(0.8)
Bile Spillage	12(9.7)
GB Perforation with Stone Spillage	10(8.1)
Injury to Adjacent Organ	2(1.6)
Converted to Open Cholecystectomy	2(1.6)
Postoperative Complication	
No Complication	123(100)
*Parenthesis indicates percentage	

Table 4. Culture outcome in relation to demographic and intra-operative parameters

Factors		Culture		P-value[b]
		Positive*	Negative*	
Gender	Male	5(17.2)	24(82.8)	0.637
	Female	20(21.3)	74(78.7)	
Age (Years)		52.71±11.97[a]	47.52±11.71[a]	0.985
Co-morbidity	Present	8(32)	17(68)	0.104
	Absent	17(17.3)	81(82.7)	
Bowel Adhesion	Yes	4(23.5)	13(76.5)	0.724
	No	21(19.8)	85(80.2)	
Omental Adhesion	Yes	9(25)	27(75)	0.407
	No	16(18.4)	71(81.6)	
Calot's triangle frozen	Yes	5(35.7)	9(64.3)	0.129
	No	20(18.5)	89 (81.7)	
GB wall inflammation	Yes	3(30)	7(70)	0.428
	No	22(19.5)	91(80.5)	
Mucocele	Yes	0 (0)	3(100)	0.376
	No	25(20.8)	95(79.2)	
Pyocele	Yes	1(19.8)	1(80.2)	0.293
	No	24(50)	97(50)	
Bile Spillage	Yes	2(16.7)	10(83.3)	0.740
	No	23(20.7)	87(79.3)	

*Parenthesis indicates percentage
[a]Mean ±standard deviation
[b]P< 0.05 was considered statistically significant

On analysis of positive bile samples, most commonly isolated organisms were *E.coli, Klebsiella spp, Pseudomonas spp,* and concomitant infection of *E.coli and Klebsiella Pneumoniae*. Their sensitivity to commonly used prophylactic antibiotics and resistance pattern was determined. Overall, for *E.coli, Klebsiella Pneumoniae* and *Pseudomonas spp.*, sensitivity to amikacin, gentamicin, clindamycin, meropenem, imipenem, linezolid, cefotaxime, piperacillin + tazobactum was found with variable resistance encountered against ampicillin and ceftriaxone. It also shows that sensitivity profile is lower in cases of concomitant growth of 2 bacterial species than those with single bacterial growth (Table 5).

Table 5. **Sensitivity pattern of antibiotics used against isolates from positive culture samples**

Antibiotics	Sensitive				Resistant			
	E. coli n (%)	*Klebsiella Spp* n (%)	*Pseudomonas Spp* n (%)	*Ecoli, K. Pneumoniae* n (%)	*Klebsiella Spp* n (%)	*E. Coli* n (%)	*Pseudomonas Spp* n (%)	*Ecoli, K. Pneumoniae* n (%)
Amikacin	11(44)	2(8)					1(4)	1(4)
Ampicillin					1(4)	5(20)	2(8)	1(4)
Cefepime	2(8)							
Cefotaxime	8(32)					3(12)		
Ceftriaxone	2(8)					2(8)	1(4)	
Clindamycin	9(36)							
Cotrimoxazole				2(8)				
Ciprofloxacin	1(4)							
Doxycycline	1(4)							
Gentamicin	12(48)						1(4)	
Imipenem	5(20)	2(8)		2(8)		1(4)		
Levofloxacin						1(4)		
Linezolid	3(12)	2(8)					1(4)	
Meropenem				2(8)		1(4)	1(4)	
Piperacillin + Tazobactum	6(24)		4(16)					

DISCUSSIONS

Biliary tract bile is usually sterile in absence of gallstones or any other biliary tract disease. Gallstones or biliary tract obstruction increases the prevalence of bactibilia.[8] Bactibilia may increase the risk of adverse post-operative outcomes following laparoscopic cholecystectomy.[3,8,9] The primary finding of our study was that the incidence of bactibilia in patients with cholelithiasis who underwent laparoscopic cholecystectomy was 20.33%. On reviewing the literature, bactibilia identified in different studies was 16-54%.[7,10] While, the rate of bactibilia in patients with symptomatic gallstone disease and chronic cholecystitis ranges from 11% to 30%, which is comparable our study.[6,8] A study by Dongol et al. showed 20% of positive bile culture samples, similar to our study where, it is 20.33%.[11]

Various studies present Gram-negative aerobes as most frequently isolated organisms from bile in symptomatic gallstones disease.[1,2,12] In a retrospective study analysis, Gram-negative bacteria (76.8%) were predominant microorganisms, which was similar to our study, followed by Gram-positive bacteria (22.5%).[13] However, one study from Germany showed that among all the bacterial isolates from bile culture, more were Gram-positive (57%), and Enterococcus species were predominant (33%), contrary to the results in our study.[14] In Suri et al. study, *Escherichia coli* (53.84%) was one of the most common isolated bacteria followed by *Pseudomonas aeruginosa* (26.92%) and Staphylococcus aureus (19.23%).[15] Similarly, in Abeysuriya et al. study, *E.coli* isolates predominantly, followed by *Pseudomonas aeruginosa, Enterococcus spp, Klebsiella spp and S.epidermidis.*[2] In our present study as well, *E.coli* (14.63%) was the most common organism isolated. Other organisms; *Pseudomonas* and *Klebsiella* were also present which agrees with many previously published studies. A Capoor et al. study, monomicrobial infection was observed in 32 patients (30.8%) and polymicrobial infection of *P. aeruginosa* with *Klebsiella* pneumonia in 4 patients (3.8%).[16] Whereas, in our study, polymicrobial infection of *E.coli* with *Klebsiella* was observed in 2 patients (1.62%).

In a study conducted at Armed Forces Medical College and Command Hospital Complex, Pune, mean age of 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.[3] In our present study, majority of patients were found in age group of 50-60 years (38 patients; 30.8%) with predominance of female (94 patients; 76.4%) comparable to other studies. A study showed that age greater than 60 years had a significant influence on the rate of positive bile cultures while sex and number of stones in gallbladder had no any influence.[17] Similarly, a study has showed statistically significant association between positive bile culture and patient's age and presence of associated co-morbidities.[1] In our present study, positive bile culture was reported highest in mean age group of 52.71 ± 11.97 and in those with co-morbidities (8 patients, 32%).

The wound infections after laparoscopic cholecystectomy was reported 5.3%, however there was no correlation between positive bile culture and wound infection.[18] Similarly, another study reported that the positive bile culture has no relationship with demographic and clinical factors and no significant effect on the occurrence of

post-operative complications.[12] But, a study showed post-operative wound infections being more common (15.79%) in patients with positive bile samples demonstrating strong correlation between bile culture and wound culture.[8] Darkahi et al. reported that a positive bile culture was the only factor significantly associated with the risk of post-operative infections in a multivariable logistic regression analysis.[19] Amidst the conflicting views in incidence of post-operative complications with positive bile samples, in our present study, there were no any post-operative complications in any patients whether bile culture was positive or negative.

The role of prophylactic antibiotics in laparoscopic cholecystectomy in low-risk patients is controversial as some studies recommend prophylaxis, particularly for high-risk patients only, which would reduce prescriptions of antibiotics by 60% and would have the combined effects of reducing cost, lessening the occurrence of adverse drug effects and reducing the development of antibiotic resistance.[5,9] In a study, comparison of the incidence of post-operative infections prior to and following an institutional practice of withholding prophylactic antibiotics for elective laparoscopic cholecystectomy was done which showed 3.0% infection in the study group compared to 0.9% in the controls. Based on the minimal difference reported, the routine use of prophylactic antibiotics was not supported.[20] Meanwhile, some studies have shown that prophylactic antibiotics in laparoscopic cholecystectomy decrease the incidence of post-operative complications.[8]

According to bile culture sensitivity, in various studies, imipenem, meropenem, ertapenem, tigecycline, amikacin, streptomycin, tobramycin, linezolid and piperacillin-tazobactum showed good sensitivity against isolated organisms and may be used as the first line prophylaxis in laparoscopic cholecystectomy.[3,4,7,8,10,21] A study has shown that Gram-negative bacilli are less susceptible to ceftriaxone, quinolones, and ampicillin, and the obtained results in our study are consistent with it in which resistance is encountered highly against ampicillin and ceftriaxone.[21,22] In a research study, amikacin was an effective antibiotic for *Escherichia coli*, *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Enterobacter cloacae*. Within the group of Gram-positive bacteria, the *Enterococcus* bacteria exhibited a higher level of susceptibility to linezolid, minocycline, carbapenems and tigecycline.[21] In our present study, high sensitivity was demonstrated with amikacin, meropenem, imipenem, gentamicin, clindamycin, cefotaxime, linezolid and piperacillin + tazobactum, comparable to other studies. In a hospital based prospective observational study from Pune, India, out of 39 patients in whom organisms were isolated, 100% (39 out of 39) were sensitive to amikacin and meropenem.[3] Imipenem and gentamicin showed a high sensitivity of 97.44% and 94.87%, whereas 92.31% sensitive to cefotaxime and 84.61% sensitive to ceftriaxone respectively. But highest 56.41% of resistance found with ampicillin, comparable to the findings in our study.

Limitation of our present study was small sample size, involving only one institution that compromises the ability to compare results between different organizations. In order to draw valid conclusion regarding type of organism isolated from bile and sensitivity pattern to antibiotics, a larger sample size is required.

CONCLUSION

The rate of positive bile culture and antibiotic sensitivity profile to commonly isolated organisms in our study is comparable to many previously published studies but also contradicting to result of few studies done in similar settings. This study concludes that while undergoing laparoscopic cholecystectomy in patients with symptomatic cholelithiasis, it is essential to determine the bacteriology and sensitivity pattern to commonly isolated organisms for rational selection of preoperative prophylactic antibiotics in order to reduce post-operative morbidity. It also highlights on importance of determining bactibilia in relation to demographic and intra-operative as well as post-operative findings in patients undergoing laparoscopic cholecystectomy.

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