

Aerobic Bacteria Associated with Symptomatic Gallstone Disease and their Antimicrobial Susceptibility in Western Nepal

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ABSTRACT:

Introduction: Gallstone disease is one of the most common disease affecting the gastrointestinal tract. Biliary tract infection results from bile stasis due to chronic obstruction, mainly (80%) gallstones. Biliary obstruction increases ductal pressure, resulting in bacterial proliferation and dissemination. Proper guidelines for appropriate use of antibiotics in managing uncomplicated and complicated gallstone disease are lacking; on the other hand, the antibiotic usage for its management cover a broad spectrum of organism which may not be required most of the times. This study aims to determine the microbiology of the bile culture and antimicrobial susceptibility in patients with symptomatic gallstone disease in our setup. **Methods:** This prospective study included patients admitted in surgery department with a diagnosis of symptomatic gallstone disease and subjected for laparoscopic or open cholecystectomy from 1st of October 2015 to 30th September 2016. The intraoperative bile of patients subjected for cholecystectomy were cultured aerobically in Blood agar and MacConkey agar. The isolates were identified and tested for their sensitivity pattern. The data were collected, entered and then analyzed using SPSS version 23. The descriptive statistics were calculated. **Results:** Of the total 259 patients, bile culture was negative in 183 patients (70.7%) and was positive in only 76 patients (29.3%). Pseudomonas was the most common cultured organism in 52 (68.4%) patients. Other isolated organisms included E. coli, Staphylococcus, Klebsiella, Enterococci, and Acinetobacter. Imipenem and amikacin were the most effective prophylactic antibiotics. **Conclusion:** Bile culture was negative in majority of patients with symptomatic gallstone disease. Few patients are positive in culture with predominantly Pseudomonas growth, mostly sensitive to amikacin or imipenem.

Keywords: bacteria • bile • cholecystectomy • gallstone • microbial sensitivity tests

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INTRODUCTION:

Gallstone disease is common worldwide, and its prevalence has geographical and ethnic variations. The lowest prevalence is seen in Africans. The prevalence of gallstone disease in the United States was 7.9% in men and 16.6% in women.¹ Europe has a prevalence of 5% to 15%. In Asian countries, the prevalence of gallstone disease ranges from 3% to 10%.² According to recent studies, the prevalence of gallstone disease was 3.2% in Japan,³ 10.7% in China,⁴ 7.1% in Northern India,⁵ and 5.0% in Taiwan.⁶ The incidence of disease increases with age.⁷ Cholelithiasis is an important cause of morbidity throughout the world.⁸ The incidence of symptomatic cholelithiasis is reported to be 2.2 per 1000 USA population with more than 500,000

cholecystectomies performed yearly.⁹

Biliary tract is normally sterile. Infection is a result of biliary stasis secondary to chronic obstruction. Majority of obstruction is by gallstone. Obstruction increases ductal pressure, resulting in bacterial proliferation and dissemination, the most common being gram negative bacteria.¹⁰ Ascending infection from duodenum and hematogenous route from the hepatic portal vein are two major source for bacterial invasion to biliary tract. Healthy individuals lack bactobilia owing to flushing out of organism by daily excretion of bile, but the percentage of bactobilia increases to 3% in patients with gallstones and to 30% in patients with common duct stones.^{11,12}

Biliary infection can be found in a sizable proportion in patient with gallstone. It accounts for 20-46% in patient who undergoes cholecystectomies and post-operative infection rate is 7-20%.¹³ Biliary infection can be due to gram negative, gram positive or anaerobic organisms.¹⁴ Most common cultured organisms from bactobilia are *Escherichia coli* (*E. coli*), *Enterococci*, *Klebsiella*, *Pseudomonas* and some rare organisms. Many antibiotics are used empirically for prophylaxis against post operative infection. Majority are sensitive to amoxicillin/clavulanic acid (94.4%), amikacin (90.7%) and to teicoplanin (98.3%).¹⁵

There is relatively very few national and international article showing the prevalence of the infection in bile of patients undergoing cholecystectomy.¹⁶ The conservative and prophylactic treatment for management of gallstone disease is therefore based on best guess.¹⁷ This study determines the current trend of bacteriology and their sensitivity to common antibiotics in our population with symptomatic cholelithiasis. The results obtained from a sample population help in proper use of empiric antibiotic in complicated gallstone disease till blood culture is available or use of prophylactic antibiotic for patient subjected for hepatobiliary surgery. The findings of this study will be shared with surgeons and general practitioners in different parts of the country to help them use the proper antibiotic to patients with symptomatic cholelithiasis before referring them to tertiary care, thereby reducing the morbidity associated with the disease.

METHODS:

This observational cross sectional study was carried out at department of Surgery, Lumbini Medical College Teaching Hospital, Nepal. Duration

of study was one year from 1st Oct 2015 to 30th Sept 2016. Non-probability (consecutive) sampling technique was used and a total of 259 patients were included in study. This sample size was calculated by using 20% prevalence of bacteria,¹⁷ 95% level of confidence and 5% margin of error. All patients with symptomatic cholelithiasis, 18 years or older of either gender, admitted and planned for laparoscopic or open cholecystectomies were included in the study. The patients with acute cholecystitis (severe right upper quadrant pain with pyrexia and leucocytosis, 12000-15000 cells/ μ L); obstructive jaundice (raised alkaline phosphatase > three times upper limit of normal); common bile duct stone (on Ultrasonography); already receiving antibiotics (from history); patient with other co-morbid infection, were excluded from the study to reduce bias in the study results. The approval for the study was obtained from the institutional review committee (IRC-LMC).

All patients with symptoms (pain over right hypochondrium, dyspepsia) were admitted from out-patient clinic of surgical unit for laparoscopic or open cholecystectomies. The diagnosis was confirmed on ultrasonography (showing distended gall bladder with calculi). Routine investigation like complete blood count; blood urea, creatinine, sugar; serum electrolytes and investigations for anesthesia fitness like chest X-ray, ECG and liver function tests were performed. An informed consent was obtained after explaining the purpose and procedure of the study. The patients were operated through open or laparoscopic cholecystectomy on the elective list by a single consultant surgeon. All patients were given an intravenous injection of Amikacin 500 mg at induction of anesthesia and no antibiotics were given postoperatively. After gaining access to the abdomen (laparoscopic or open), and recording the findings, bile was aspirated from gall bladder at body in a 10 ml disposable syringe and kept in a sterile container provided from microbiology department for culture and sensitivity. Gall bladder was removed after ligation and cutting of the cystic artery and duct. The collected specimen of the bile was labeled and sent to a single laboratory in sterile container. Aerobic cultures of specimen were performed for microorganisms such as *E. coli*, *Klebsiella*, *Pseudomonas*, *Acinetobacter*, *Enterococcus* and *Staphylococcus* under the supervision of expert microbiologist. For aerobic culture, the sample was inoculated on blood agar and MacConkey agar medium and incubated at 37°C for 16 to 18 hours

overnight. The culture plate was checked next day for bacterial growth. Bacterial growth was checked for Gram staining, catalase and coagulase positivity was checked for gram positive organism, biochemical test was performed for gram negative organism, oxidase test was also performed for non-lactose forming gram negative organism. Once detected the sensitivity of these bacteria was checked for antibiotics like ceftriaxone, cefoperazone-sulbactam, amikacin, ciprofloxacin, amoxy-clavulanic acid, imipenem and cotrimoxazole. Patient demographics like age, age-group, gender, bacterial culture and sensitivity reports of bile were recorded in a structured proforma. The data were analyzed with SPSS version 23 for windows. Frequency and percentages were calculated from categorical variables while mean and standard deviation was calculated for continuous variables. Common isolated bacteria were stratified among the age and sex to see the effect modifiers and cross tabulation was used to see the sensitivity pattern of common bacteria to tested antibiotics. The

data was presented in the tabular form.

RESULTS:

The total number of patients presenting with symptomatic cholelithiasis were 259. Out of these, male and female patients were 64 (24.71%) and 195 (75.29%) respectively with M:F ratio of 1:3. The mean age of male and female patients with symptomatic cholelithiasis were 46.03 yr ($SD=15.9$) and 40.67 yr ($SD=16.1$) respectively with an overall mean age of 42 yr ($SD=16.2$).

On culture and sensitivity test, 76 (29.34%) specimens had positive growth while 183 (70.66%) has no growth. The most common bacteria isolated was *Pseudomonas* in 52 (68.42%) patients. More than half ($n=43$, 56.76%) of the patients, who had a positive growth in their bile, were under 40 years of age at presentation. Full detail of age wise distribution of microbial growth is shown in Table 1. According to gender, *Pseudomonas* was isolated in 41 (78.85%)

Table 1: Age wise distribution of common bacterial isolates on culture and sensitivity of bile

Age in group (years)	Organism isolated in aerobic growth, n (%)						Total
	<i>Pseudomonas aeruginosa</i>	<i>E. coli</i>	<i>Klebsiella</i>	<i>Acinetobacter</i>	<i>Staphylococcus aureus</i>	<i>Enterococcus</i>	
18-30	16 (66.7%)	3 (12.5%)	0	0	4 (16.7%)	1 (4.2%)	24 (100%)
31-40	11 (57.9%)	3 (15.8%)	2 (10.5%)	1 (5.3%)	1 (5.3%)	1 (5.3%)	19 (100%)
41-50	8 (66.7%)	3 (25%)	0	0	0	1 (8.3%)	12 (100%)
51-60	6 (100%)	0	0	0	0	0	6 (100%)
>60	11 (73.3%)	1 (6.7%)	1 (6.7%)	1 (6.7%)	1 (6.7%)	0	15 (100%)
Total	52 (68.4%)	10 (13.2%)	3 (3.9%)	2(2.6%)	6 (7.9%)	3 (3.9%)	76 (100%)

Table 2: Sensitivity and resistance of common isolated bacteria to various antibiotics.

Sensitive Antibiotic	<i>Pseudomonas</i> $n=52$ (68.4%)		<i>E. coli</i> $n=10$ (13.2%)		<i>Klebsiella</i> $n=3$ (3.9%)		<i>Actinetobacter</i> $n=2$ (2.6%)		<i>Staph. aureus</i> $n= 6$ (7.9%)		<i>Enterococcus</i> $n=3$ (3.9%)	
	S	R	S	R	S	R	S	R	S	R	S	R
Ceftriaxone	46 (88.5)	6 (11.5)	9 (90)	1 (10)	3 (100)	0	2 (100)	0	4 (66.7)	2 (33.3)	2 (66.7)	1 (33.3)
Cefoperazone + Sulbactam	50 (96.2)	2 (3.9)	10 (100)	0	3 (100)	0	2 (100)	0	5 (83.3)	1 (16.7)	2 (66.7)	1 (33.3)
Amikacin	51 (98.1)	1 (1.9)	9 (90)	1 (10)	3 (100)	0	2 (100)	0	3 (50)	3 (50)	1 (33.3)	2 (66.7)
Ciprofloxacin	49 (94.2)	3 (5.8)	7 (70)	3 (30)	2 (66.7)	1 (33.3)	1 (50)	1 (50)	4 (66.7)	2 (33.3)	2 (66.7)	1 (33.3)
Amoxy-clavulanic acid	30 (57.7)	22 (42.3)	6 (60)	4 (40)	0	3 (100)	1 (50)	1 (50)	5 (83.3)	1 (16.7)	3 (100)	0
Imipenem	51 (98.1)	1 (1.9)	10 (100)	0	3 (100)	0	2 (100)	0	6 (100)	0	3 (100)	0
Cotrimoxazole	12 (23.1)	40 (79.9)	8 (80)	2 (20)	2 (66.7)	1 (33.3)	0	2 (100)	5 (83.3)	1 (16.7)	3 (100)	0

female and 11 (21.15%) male, *E. coli* was isolated in nine (90%) female and one (10%) male, *Klebsiella* in three (100%) female but none in male. On culture and sensitivity test, *Pseudomonas* showed high sensitivity to amikacin and imipenem (98.8% in both) and least sensitive to cotrimoxazole (23.08%). *E. coli* showed high sensitivity to imipenem and cefoperazone-sulbactam (100% each) and least to amoxy-clavulanic acid and ciprofloxacin (60% and 70% respectively). Sensitivity and resistance of these six bacteria to various antibiotics is shown in detail in Table 2.

DISCUSSION:

Our study showed positive culture of bile in 76 (29.34%) patients presenting with symptomatic cholelithiasis, which was comparable to other study with rates of 16-54%.^{13,18-20}

The most common bacteria isolated in our study was *Pseudomonas* in 52 patients (68.42%), *E. coli* in 10 patients (13.16%), *Staphylococcus* in six patients (7.89%), *Klebsiella* and *Enterococci* in three patients each (3.95%), and *Acinetobacter* in two patients (2.63%). This is in contrast to the study by Capoor et al. where the most common organisms isolated were *Escherichia coli* ($n=11$, 29.7%), *Klebsiella pneumoniae* ($n=10$, 27%), *Citrobacter freundii* ($n=3$, 8.1%), *Salmonella enterica* serovar Typhi ($n=3$, 8.1%), *Pseudomonas aeruginosa* ($n=2$, 5.4%), *Acinetobacter* spp. ($n=1$, 2.7%), *Candida krusei* ($n=1$, 2.7%), *Staphylococcus aureus* ($n=1$, 2.7%). Polymicrobial infection of *P. aeruginosa* with *K. pneumoniae* was observed in four patients (3.8%).¹¹

Pseudomonas aeruginosa has been reported as the predominant flora by Dhil et al.²¹ In a study by Ozturk et al.²² 114 patients who underwent cholecystectomy for various reasons showed bacterial growth in the bile culture of 15 patients (13.1%). The most commonly isolated bacteria were *Enterococcus* spp (four patients, 26.6%), *Escherichia coli* (three patients, 20%) and *Enterobacter* spp (three patients, 20%). The bile culture positivity rate was highest in patients with acute cholecystitis combined with choledocolithiasis (three patients, 100%). The bile culture bacterial growth was highest in patients over 60 years of age (10 patients, 27 %) and in those with concomitant illness (nine patients, 23.6%).

Another study showed *E. coli* (30%), *Enterobacter* sp. (15%), *Staphylococcus aureus* (10%), *Streptococcus faecalis* (15%), *Klebsiella* (5%), *Serratia* (2.5%), *Streptococcus* (2.5%),

Streptococcus sp (20%) in growth from gallbladder bile culture.²³ A study in aspirated bile specimen from common bile duct in 150 patients with hepatolithiasis who underwent surgical intervention showed bacteria in all patients. The bacteria most frequently found were gram-negative bacteria such as *Klebsiella* sp, *Escherichia coli*, and *Pseudomonas* sp. Gram positive bacteria like *Enterococcus* sp. and anaerobes like *Bacteroides* sp were also found.²⁴

Abeyasuriya et al. performed a case control study of 70 bile samples and revealed that *E. coli* was the most common growth, followed by *P. aeruginosa*, *Enterococcus* spp, *Klebsiella* spp. and *S. epidermidis*. There were no bacterial isolates in the bile of controls.¹³ In a study done by Ahmad et al., with 268 cases operated by open or laparoscopic cholecystectomy for symptomatic cholelithiasis, 157 (58.58%) have positive growth. The most common bacteria were *Escherichia coli* in 69 (25.74%), *Klebsiella* in 46 (17.16%), *Salmonella* in 34 (12.68%), and *Shigella* in 17 (6.34%) patients. On culture and sensitivity test, all the four isolated bacteria showed sensitivity to cefuroxime, ceftriaxone and ciprofloxacin in more than 50% cases, while all the four bacteria showed resistance to amoxicillin in more than 50% cases.²

Study by Ahmaad Maqsood et al. in 106 patients with cholecystectomy for symptomatic gallstones showed positive bile culture in only 25 patients (23.6%). *E. coli* was the most common cultured organism in 10 (40%) patients, *Klebsiella* in five (20%) patients, *Pseudomonas* in five (20%) patients, *Proteus* in two (8%) patients, *Staphylococcus aureus* in two (8%) patients, and mixed organisms were cultured in one patient (4%). Cefoperazone with sulbactam and amikacin were the most effective antibiotics.²⁵

Although surgery remains the gold standard therapy for symptomatic cholelithiasis and its complications, a period of hospitalization is required before elective or emergency cholecystectomy. Our patients also received the empirical prophylactic antibiotic before the surgical procedure according to recommended guidelines.^{26,27} In our study, on culture and sensitivity test, *Pseudomonas* showed high sensitivity to amikacin and imipenem (98.8% in both) and least sensitive to cotrimoxazole (23.08%). *E. coli* showed high sensitivity to imipenem and cefoperazone-sulbactam (100% each) and least to amoxy-clavulanic acid and ciprofloxacin (60% and 70% respectively). It seems that history of previous and recurrent hospitalization, prolong

hospital stay and wide spread use of broad spectrum antibiotics has led to the selective survival and emergence of resistant organism.^{28,29} Therefore, antimicrobial activity against potential causative organisms, the severity of the cholecystitis, and the local susceptibility pattern must be taken into consideration when prescribing drugs.

CONCLUSION:

The most common bacteria of symptomatic cholelithiasis isolated were *Pseudomonas* followed

by *E. coli*, *Klebsiella*, *Acinetobacter*, *Staphylococcus*, and *Enterococcus*. Most of these organisms were sensitive to imipenem and amikacin. The empirical antibiotics used for the treatment of symptomatic cholelithiasis must cover these common bacteria. Imipenem and amikacin must be a part of empirical regime as it will help in reducing the morbidity associated with symptomatic cholelithiasis.

Conflict of interest: None declared.

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