

Microbiological profile of bile sample in patients undergoing cholecystectomy at a tertiary care centre

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ABSTRACT

Introduction: Cholecystectomy is a frequently performed operation for symptomatic cholelithiasis. The presence of gallstones is associated with the bacterial colonization of the bile.

Objectives: The objective of this study is to access the pattern of bacterial isolates from bile aspirates from cholecystectomy patients and also to determine antibiogram of isolated organisms.

Methodology: A descriptive cross-sectional study was conducted among 140 patients who underwent cholecystectomy from 1st October 2024 to 31st December 2024 at Kathmandu Medical College and Teaching Hospital after approval from Institutional Review Committee. Bile samples received from all the patients who underwent cholecystectomy were included in this study while samples from other illness were excluded. Intra operatively, bile was aspirated from gall bladder and sent for culture and antibiotic sensitivity testing.

Result: A total of 33(24%) patient's bile was culture positive. *Escherichia coli* 18 (54%) was the most common isolates followed by *Pseudomonas aeruginosa* 6(18%), *Klebsiella pneumoniae* 4(12%), *Klebsiella oxytoca* 3(9%) and *Acinetobacter baumannii* 2(6%) respectively. Most of the isolates were sensitive to Amikacin, piperacillin /Tazobactam and Meropenem.

Conclusion: *Escherichia coli* was the most common isolates followed by *Pseudomonas aeruginosa*. Amikacin, Meropenem and Piperacillin/Tazobactam were sensitive to most of the organisms except for *Acinetobacter baumannii* which showed resistant to these antibiotics.

Keywords: bile culture; cholecystectomy; prophylactic antibiotics.

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INTRODUCTION

The most common reason for cholecystectomy is cholelithiasis. The presence of gallstones in the gallbladder or biliary tree is associated with the bacterial colonization of the bile. The incidence of bactibilia is low in uncomplicated cholelithiasis, whereas it is higher in complicated cholelithiasis cases due to obstruction and stasis.¹ Microbial flora in disease associated with gallbladder has been detected in up to 80% of patients presenting with acute symptoms and in >25% patients with chronic disease of gallbladder.^{2,3} Gram negative bacteria are more commonly associated with GB disorders.⁴⁻⁶

Antibiotic policy and appropriate antibiotic prophylaxis cannot be designed unless the data is available about bacteria colonizing the bile associated with gall bladder disease. Objective of this study was to access the pattern of bacterial isolates from bile aspirates of cholecystectomy patients seeking care at a tertiary care teaching hospital.

METHODOLOGY

A descriptive cross-sectional study was conducted within a period of three months from 1st October 2024 to 31st December 2024 among the patients undergoing cholecystectomy at the Department of Clinical Microbiology laboratory Sinamangal, Kathmandu Medical College and Teaching Hospital (KMCTH). Ethical approval was obtained from the Institutional Review Committee (Reference Number:12082024/09) before this study was conducted.

Convenience sampling was done and the sample size was calculated from study done in Nepal with culture positive rate of 23.3%⁷. Bile samples received from all the patients who underwent cholecystectomy were included in this study and bile samples from other illness were excluded. The sample size was calculated using formula,

$$P = 23.3\%$$

$$n = z^2 pq / e^2$$

$$= (1.96)^2 \times 23.3 \times 76.7 / 7^2 = 140$$

Where,

n= minimum required sample size

Z= 1.96 at 95% Confidence Interval (CI)

p= past prevalence

q= 1-p

e= margin of error, 7%

The patients were admitted a night before surgery in the department of surgery and were examined and evaluated by concerned surgical team and anaesthetic team for fitness for surgery. Informed consent was taken from the patient or patient's attendant after explaining the nature of procedure such as open or laparoscopic, and even potential for conversion from laparoscopic approach to open cholecystectomy. All the patients were given single dose of IV injection of Ceftriaxone 1 gram at induction of anaesthesia and 2 doses of the same were given postoperatively. Five ml of bile samples were collected in a sterile container with the help of 5ml disposable syringe. Container was labelled and sent to the laboratory without any delay. Bile collected was cultured on Blood agar (BA) and MacConkey agar (MA), which was aerobically incubated at 37°C for 24- 48 hours for the growth of organisms. The bile culture was reported sterile when there was no growth even after 48 hours of incubation. All positive culture was identified

by their colony morphology on the plate, microscopic examination of Gram's staining and appropriate biochemical reactions. Antimicrobial susceptibility testing was performed on Muller Hinton agar plates using Kirby Bauer disc diffusion method according to Clinical and Laboratory Standard Institute (CLSI) Guidelines.⁸ Data analysis was done by using statistical package for social sciences, IBM SPSS Statistics for Windows version 19 (IBM Corp., Armonk, N.Y., USA).

RESULTS

Out of total 140 patients 33 (23.5%) male and 107 (76.42%) females were included in this study. Maximum number of patients 39 (27%) were from the age group 41-50 years followed by 34 (24%) from the age group of 31-40 years. (Figure 1)

Out of 140 bile samples received for microbiological analysis, 33 (24%) were culture positive. *Escherichia coli* 18 (54.54%) was the most common isolates followed by *Pseudomonas aeruginosa* 6 (18%), *Klebsiella pneumoniae* 4 (12.12%), *Klebsiella oxytoca* 3 (9%) and *Acinetobacter baumannii* 2 (6%) respectively. Gram positive organisms were not isolated during this study. The remaining bile sample were sterile 107(76%, Table 1).

On antibiogram, *E. coli* showed maximum sensitivity to Meropenem 17(94.4%) followed by Amikacin in 15 (83.3 %) whereas maximum resistance was shown by Cefixime 13 (72%) followed by amoxicillin-clavulanate 8 (44%).

All 6 strains of *Pseudomonas aeruginosa* exhibited 100% sensitivity towards Piperacillin Tazobactam and 66% towards Amikacin and Meropenem. Three i.e. half of the strains were resistant to Ciprofloxacin and Ceftazidime (Table 3).

Among the *Klebsiella pneumoniae* isolates, 3(75%) were sensitive to Amikacin and 100% were resistant to Cefixime and Amoxyclav. Whereas *Klebsiella oxytoca* showed only 33% sensitive towards Amikacin, Cefixime, Ceftriaxone, Amoxyclav, piperacillin/Tazobactam and Meropenem and were 3(100%) resistant towards Ciprofloxacin.

Out of 2 *Acinetobacter baumannii*, both showed 100% sensitivity towards Tigecycline and Polymyxin B whereas (50%) were only sensitive to Amikacin, Piperacillin/ Tazobactam and Meropenem.

Table 1: Microbiological profile of bile sample

Culture report	n (%)
Positive	33 (24)
Sterile	107 (76)
Isolated organism from bile sample (n=33)	
<i>Escherichia coli</i>	18 (54.54)
<i>Pseudomonas aeruginosa</i>	6 (18.18)
<i>Klebsiella Pneumoniae</i>	4 (12.12)
<i>Klebsiella Oxytoca</i>	3 (9)
<i>Acinetobacter baumannii</i>	2 (6)

Table 2: Antibiotic sensitivity pattern of *Escherichia coli* (n = 18)

Antibiotics	Sensitive n (%)	Resistant n (%)
Amikacin	15 (83.33)	3 (16.66)
Cefixime	5 (27.7)	13 (72)
Ceftriaxone	7 (38.88)	11 (44.4)
Ciprofloxacin	10 (55.55)	8 (44.4)
Amoxicillin-clavulanate	10 (55.55)	8 (44.4)
Piperacillin/Tazobactam	13 (72.22)	5 (27.77)
Meropenem	17 (94.4)	1 (5.55)

Table 3: Antibiotic sensitivity pattern of *Pseudomonas aeruginosa* (n = 6)

Antibiotics	Sensitive n (%)	Resistant n (%)
Amikacin	4 (66.66)	2 (33.33)
Ceftazidime	3 (50)	3 (50)
Ceftazidime/Clavulanic acid	4 (66.66)	2 (33.33)
Ciprofloxacin	3 (50)	3 (50)
Piperacillin/Tazobactam	6 (100)	0 (0)
Meropenem	4 (66.6)	2 (33.3)

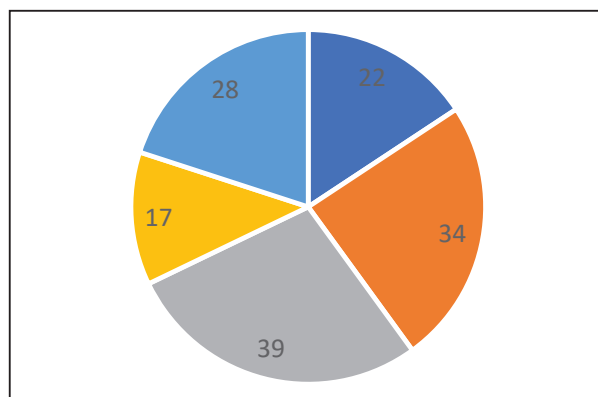


Figure 1: Distribution of patients according to age.

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.⁹ Around 94% of biliary infections are caused by aerobic organisms, while anaerobic organisms account for the rest.¹⁰ 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.¹¹ 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 bacterial colonisation in bile results in gallstone formation or vice-versa, but bacterial biofilm covers 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. This study was conducted to access the pattern of bacterial isolates from bile aspirates among cholecystectomy patients and also to determine their antibiogram.

In the present study out of 140 bile samples received, 33(24%) were culture positive and remaining 107(76%) were sterile which is similar to the study conducted by Patel V. et al with positive bile cultures of 19(24.36%) and those with negative culture were 59(75.64%).¹² In contrast to our study, Shankaran et al reported 14.66% (39 out of 266) positive growth and 15.6% in a study done by Morris et al.^{13,14} *E. coli* was the most common organism isolated in our study which was 54% among bile culture positive and 12 % among all patients followed by *Pseudomonas aeruginosa* 4%, *Klebsiella pneumoniae* 2.8%, *Klebsiella oxytoca* 2.1% and *Acinetobacter baumannii* 2% among all samples respectively. This study is in accordance to the study done by Patel V. et al where *E. coli* isolated were (63.16%) and 15.38% among all patients followed by *Pseudomonas* 3.85%, *Klebsiella* 2.56%, *Staphylococcus Viridans* (1.28%).¹²

E. coli showed good sensitivity to Meropenem(94%) and amikacin but was resistant to Cefixime (72%). In a study done by Ahmed et al, *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.¹⁵

Pseudomonas aeruginosa exhibited 100% sensitivity towards Piperacillin /Tazobactam and 66% towards Amikacin and Meropenem. 3(50%) isolates were resistant to Ciprofloxacin and ceftazidime.

Among the *Klebsiella pneumoniae* isolates, 3(75%) were sensitive to Amikacin and 100% resistant to Cefixime and Amoxyclav. Whereas *Klebsiella oxytoca* showed only 33% sensitive towards Amikacin, Cefixime, Ceftriaxone, Amoxyclav, piperacillin/Tazobactam and Meropenem and were 3(100%) resistant towards Ciprofloxacin.

Out of 2 *Acinetobacter* isolates, both showed 100% sensitivity towards Tigecycline and Polymyxin B whereas (50%) were only sensitive to Amikacin, piperacillin/Tazobactam and Meropenem. There were few limitations. We could not perform anaerobic culture due to limited resource at our center.

In present study 33(24%) were culture positive . There is conflicting evidence in various literatures about the association of positive bile culture with postoperative infective complications. However, number of studies has shown relationship of positive bile culture and infective complications like fever, wound infection and sepsis^{1,16-17}

CONCLUSION

Cholecystectomy was the surgery commonly done among the female patients. Maximum number of patients were from the age group 41-50 years. *Escherichia coli* was the most common isolates followed by *Pseudomonas aeruginosa*. There was variation in antibiotic sensitivity testing depending upon the organisms. Amikacin, Meropenem and Piperacillin/Tazobactam were sensitive to most of the organisms except for *Acinetobacter baumannii* which showed resistant to these antibiotics. Thus, this study can help to develop local guidelines and recommendations based on Nepalese data to ensure the rational use of prophylactic antibiotics in patients with symptomatic cholelithiasis undergoing for cholecystectomy.

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