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Original Article

Surgery Section

Bile Culture in Patients Undergoing Cholecystectomy: A Cross-sectional Study

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ABSTRACT

Introduction: Due to the blockade of gallstones, the hepatobiliary system, especially the gall bladder, can undergo chemical inflammation called cholecystitis. It is mostly complicated by the invasion of gut bacteria.

Aim: To evaluate the incidence of biliary microflora and prepare an antibiogram for all the gall bladder pathologies in a tertiary care hospital in South India.

Materials and Methods: The current study was a cross-sectional study carried out at a tertiary care hospital in Chennai, Tamil Nadu, India, among patients undergoing cholecystectomy from March 2024 to February 2025. Bile was collected from each patient and sent for culture and antibiogram. The data were recorded into Microsoft Excel and the statistical data analysis was carried out using Statistical Package for Social Sciences (SPSS) version 26.

Results: A total of 60 patients were enrolled for this study. Out of 60, 27 (45%) specimens showed no growth, 11 (18.3%) were positive for *Escherichia coli (E. coli)*, 9 (15%) for *Klebsiella*, 6 (10%) for *Proteus*, 4 (6.7%) for *Enterococcus*, 2 (3.3%) for *Salmonella*, and 1 (1.7%) for *Shigella*. All the isolates except *Klebsiella* and *Proteus* were 100% sensitive for Piptaz. Amoxycillin was found to be least effective followed by ceftriaxone with all 6 and 3 isolates, respectively having ≥50% resistivity.

Conclusion: Biliary microflora antibiogram revealed resistivity against commonly used antibiotics including amoxicillin, ciprofloxacin and ceftriaxone while most of the isolated microbial strains were found sensitive to Piptaz and meropenem.

Keywords: Antibiogram, Cholecystitis, *Escherichia coli*, Gallstones, *Salmonella, Shigella*

INTRODUCTION

The gall bladder is a hollow, pear-shaped organ. The bile acids, as synthesised by the liver, are stored and concentrated in the gall bladder and get secreted into the second part of the duodenum [1,2]. One of the pathologies of the biliary tract is the formation of gallstones. Gallstones are predicted to be present in ten to twenty per cent of the adult population, globally [3]. Gallstones are formed either in the biliary tract or in the gallbladder. Based on their composition, they are divided into pigment stones and cholesterol stones. Being obese, being inactive, a high-carbohydrate diet, low fibre intake, and hormone replacement therapy are some of the risk factors of gall bladder formation [4,5]. Cholesterol stones are the result of failure in the bile cholesterol homeostasis. Supersaturation of the bile with cholesterol results from both excessive cholesterol secretion and hyposecretion of either phospholipids or bile salts [6]. Pigment stones are formed due to an abnormality in the bilirubin metabolism. These can be either black or brown in colour [7-9].

The most common reason for the occurrence of cholecystitis is the blockade of the hepatobiliary structure through gallstones. The block could occur at the gallbladder's neck or the cystic duct [10]. acute cholecystitis is a type of inflammation caused by chemicals such as bile salts, Ceftriaxone, and polyphenol activators. Impacted stone also causes mucosal erosion allowing bile salts to act over the submucosal tissues as bile is toxic to these tissues. It leads into necrosis, further infection and often perforation of the gallbladder usually at Hartmann's pouch [11-13]. The invasion of gut microorganisms is the main factor complicating this. The common aerobes include *Escherichia coli* (*E. coli*), *Streptococcus faecalis* and *Klebsiella*. Common anaerobes found include *Clostridia* and *Bacteroides*. Bactibilia is said to be present in 60% of all the early cholecystitis cases. The frequency was found to be higher among older individuals and also those with recurrent cholecystitis. There is a higher complication risk in patients with bactibilia [14].

This research focused on identifying and characterising the microbial strains in context of antibiotic sensitivity/resistivity in cases with bile contamination, the emergence of antibiotic-resistant bacteria, and personalized antibiotic strategies, which are still evolving and need more robust evidence. The current research was carried out to identify organisms present in the bile of post-cholecystectomy patients and to determine antibiotic sensitivity/resistivity profile in a tertiary care hospital in South India aimed to create an empirical antibiotic regime suitable for cholecystitis patients for a faster recovery. There is a need for such research to guide clinicians in targeted antibiotic use, reduce unnecessary prophylaxis, and improve outcomes.

MATERIALS AND METHODS

The present prospective observational cross-sectional study was carried out in the Department of General Surgery of a tertiary care hospital in Chennai, Tamil Nadu, India for a period of 12 months starting from March 2024 to February 2025. The study received ethical clearance from the Institutional Ethics Committee with reference number (002SBMCH/IHEC/2023/2079).

Inclusion and Exclusion criteria: All the patients with a diagnosis of gallstones were enrolled for this research. Those under 18 years, pregnant women and those not willing to give consent were excluded from this research.

Sample size calculation: The sample size was calculated using the formula $n=z^2*P(1-P)/e^2$, using prevalence as ~5% [15], precision as 0.05 and Z value as 1.96, with the final sample found to be 60. The technique used was purposive sampling.

Study Procedure

Demographic data such as age, gender, weight, height, Body Mass Index (BMI), food habits, locality and clinical condition were recorded in a semi-structured questionnaire on admission. The patients were subjected to examination,and the findings were noted. These included the presence of jaundice, as evidenced by

yellowish discoloration of the sclera, and abdominal tenderness, if present, was noted down along with the presence of Murphy's sign. Additional clinical data was recorded as type of surgery, either open or laparoscopic cholecystectomy, the type of stones and the number of stones with the classification of cholesterol stones, pigment stones, and mixed stones. Bile was collected by several means, depending on the clinical requirement. Bile was collected during laparoscopic cholecystectomy, open cholecystectomy, Endoscopic Retrograde Cholangiopancreatography (ERCP) percutaneous biliary drainage, intraoperative collection, direct aspiration during surgery (e.g., cholecystectomy) and collected from the gallbladder or bile ducts using a sterile syringe. Microbiological analysis of the bile was carried out by culture method and antibiogram.

STATISTICAL ANALYSIS

The data were recorded into Microsoft Excel and the statistical data analysis was carried out using SPSS version 26.

RESULTS

The demographics of the study population is explained in[Table/Fig-1]. Majority of the study participants were 41-50 years of age with a female preponderance. The male-to-female ratio was 2:1 in the present study. A total of 36 (60%) participants were from urban areas and 38 were consuming a mixed diet. A total of 17 (28.3%) patients were overweight and 11 (18.3%) were obese. Most of the study participants 54 (90%) reported having abdominal pain, followed by other symptoms such as fever, dyspnoea, nausea, and vomiting at the time of admission. Rest of the clinical profile of the study participants has been described in [Table/Fig-2].

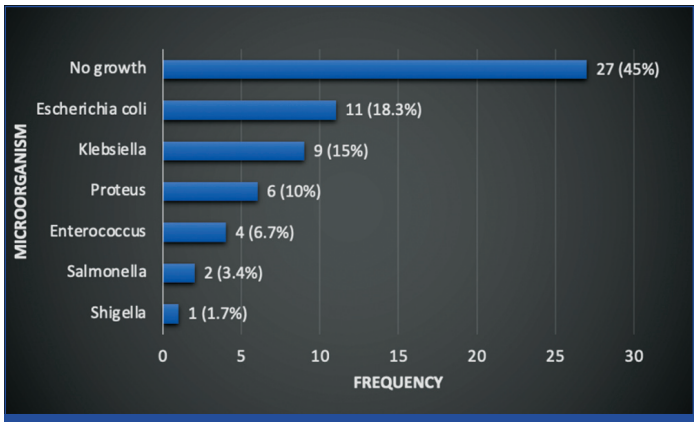
Variables		Frequency (n=60)	Percentage (%)
Age group (in years)	21-30	7	11.7
	31-40	15	25
	41-50	22	36.7
	51-60	12	20
	>60	4	6.7
Sex	Male	18	30
	Female	42	70
Locality	Urban	36	60
	Rural	24	40
Type of diet	Vegetarian	22	36.7
	Mixed type	38	63.3
BMI (Kg/m^2)	<18	7	11.7
	18-25	25	41.7
	26-30	17	28.3
	>30	11	18.3

[Table/Fig-1]: Baseline characteristics among the participants.

Microbial culture profile of the study participants showed a 11 (18.3%) positivity for *E. coli*, 9 (15%) for *Klebsiella*, 6 (10%) for *Proteus*, 4 (6.7%) for *Enterococcus*, 2 (3.3%) for *Salmonella* and 1 (1.7%) for *Shigella*. Rest 27 (45%) of the samples showed no growth [Table/Fig-3]. All the isolated *E. coli* and *Enterococcus* strains were found to be sensitive to Piptaz, 10 (90.9%) *E. coli* cultures were sensitive for meropenem and 81.8% for amikacin with 63.6%, 54.5%, and 36.4% resistivity was observed against amoxicillin, ciprofloxacin and ceftriaxone respectively. *Klebsiella* was found to be 100% sensitive to meropenem and maximum resistance was observed against amoxicillin with 77.8%. *Enterococcus* was also found sensitive to meropenem (100%) and amikacin (75%) whereas, ceftriaxone, ciprofloxacin and amoxicillin were found to have resistance in 75% and 50% each respectively. The isolated *Salmonella* showed 100% sensitivity to Piptaz and amikacin and complete resistance observed against amoxicillin. *Shigella* showed 100% sensitivity to ceftriaxone,

Variables		Frequency (n=60)	Percentage (%)
Symptoms	Abdominal pain	54	90
	Fever	23	38.3
	Dyspepsia	21	35
	Nausea/Vomiting	22	36.7
Signs	Jaundice	21	35
	Abdominal tenderness	48	80
	Murphy's sign	35	58.3
Type of stones	Yellow/Cholesterol	24	40
	Black/Pigment	36	60
Number of stones	Single	26	43.3
	Multiple	34	56.7
Type of surgery	Open cholecystectomy	21	35
	Laparoscopic cholecystectomy	39	65

[Table/Fig-2]: Clinical characteristics among the participants.



[Table/Fig-3]: Bar chart showing distribution according to biliary flora obtained.

Piptaz, meropenem and amikacin and showed 100% resistance to ciprofloxacin and amoxicillin, respectively. Detailed drug sensitivity/resistivity profile has been mentioned in [Table/Fig-4].

DISCUSSION

Gallstones are formed in either the biliary tract or the gallbladder as a result of high cholesterol or bilirubin levels in bile [3]. Due to blockage of gallstones, the hepatobiliary system, especially the gall bladder, can undergo chemical inflammation called cholecystitis, which can be acute or chronic. It is mostly complicated by the invasion of gut bacteria. The common aerobes include *Escherichia coli*, *Streptococcus faecalis* and *Klebsiella* [4,5]. Common anaerobes found include *Clostridia* and *Bacteroides*. Bactibilia is said to be present in 40-60% of all the early cholecystitis cases. The frequency was found to be higher among older individuals and also those with recurrent cholecystitis and bactibilia associated with higher complications [14]. This research aimed to evaluate the incidence of biliary microflora and prepare an antibiogram for all the gall bladder pathologies in a tertiary care hospital in South India. Age distribution of the study participants showed 36.7% patients between age-group 41-50 years, closely followed by 31-40 years age-group with 25% patients. There was a female preponderance observed with a male-to-female ratio noted as 2:1, which was in concordance to the available published literature [10,14,16]. Majority of the patients belonged to the urban regions with mixed diet. This can be attributed to ease of access to the healthcare facilities in urban population.

Abdominal pain was the most common observed clinical symptom, 38.3% had fever, 35% had dyspnoea, and 36.7% had nausea or vomiting at the time of admission. A 35% had jaundice, and 80% had abdominal tenderness. 58.3% were positive for Murphy's sign. A 28.3% were overweight and 18.3% were obese. Published literature suggests that for each 5-unit rise in BMI, the chance of

Organisms	S/R	Ceftriaxone		Piptaz		Ciprofloxacin		Amoxicillin		Meropenem		Amikacin	
		N	%	N	%	N	%	N	%	N	%	N	%
E. coli	S	7	63.6	11	100	5	45.5	4	36.4	10	90.9	9	81.8
	R	4	36.4	0	0	6	54.5	7	63.6	1	9.1	2	18.2
Klebsiella	S	6	66.7	8	88.9	4	44.4	2	22.2	9	100	5	55.6
	R	3	33.3	1	11.1	5	55.6	7	77.8	0	0	4	44.4
Proteus	S	2	33.3	5	83.3	4	66.7	3	50	6	100	4	66.7
	R	4	66.7	1	16.7	2	33.3	3	50	0	0	2	33.3
Enterococcus	S	1	25	4	100	2	50	2	50	4	100	3	75
	R	3	75	0	0	2	50	2	50	0	0	1	25
Salmonella	S	1	50	2	100	1	50	0	0	1	50	2	100
	R	1	50	0	0	1	50	2	100	1	50	0	0
Shigella	S	1	100	1	100	0	0	0	0	1	100	1	100
	R	0	0	0	0	1	100	1	100	0	0	0	0

[Table/Fig-4]: Distribution according to antibiotic sensitivity among the organisms obtained.

gallstone development increases by 1.63 times and the probability of gallstones among diabetics was reported to be 1.56 times that of non-diabetic individuals [17,18]. In this study, 60% of the patients had pigmented stones, and rest had cholesterol ones with majority having multiple stones.

A total of 45% of the specimens were negative for microbial culture. An 18.3% samples were positive for *Escherichia coli*, 15% for *Klebsiella*, 10% for Proteus, 6.7% for *Enterococcus*, 3.4% for *Salmonella*, and 1.7% for *Shigella*. Lee WJ *et al*., conducted a study with regard to the bacteriology of bile. The study reported 68% to have positive growth. The complication rate was found to be higher among those with cholangitis with aerobes (87.7%) observed in majority and only 12.3% were anaerobes. Among the aerobes, the common one was *Enterococcus*, followed by *E. coli* and *Pseudomonas* [19]. Kaya M *et al.,* reported that bile culture came out positive in ~50% of the patients. Gram-negative bacteria like *E. coli* and *Pseudomonas* have been frequently encountered organisms [20]. Moti MR *et al*., conducted a similar study and reported the common organisms to be found were Gram-positive-*Streptococcus* and Gram-negative - *Citrobacter frondeii*. Fluoroquinolones were the agent to which most organisms were reported sensitive [21]. Ballal M *et al*., reported that among the cultures grown, 56% were aerobes and 13% were anaerobes. Among the aerobes, *E. coli* was predominant, followed by *Klebsiella* and *Bacteroides fragilis* as most common anaerobe [22].

All the isolated strains of *E. coli, Enterococcus,* and *Salmonella* were sensitive to Piptaz and all strains of *Klebsiella, Proteus* species were sensitive to meropenem. Additionally, *Enterococcus,* and *Salmonella* were sensitive to meropenem and amikacin respectively. The common highest resistance was noted against amoxicillin, ciprofloxacin, and ceftriaxone. According to Kaya M et al., antibiotics to which the organisms obtained from bile culture were sensitive were meropenem, imipenem and amikacin [20]. Shafagh S et al., stated antibiograms of cultures from bile showed 100% sensitivity to Imipenem, 98.1% to amikacin and 90.4% to Gentamycin among Gram-negative organisms, while Gram-positive ones showed 100% sensitivity to imipenem, rifampicin, vancomycin and clindamycin [23]. A study from Iran reported that clindamycin, amikacin and ceftriaxone were also found to be effective against the microbial isolates obtained from bile cultures [24]. In a study by Salvador VB et al., *E. coli* showed higher sensitivity to amikacin, ceftriaxone, cefepime, Piptaz, gentamycin, meropenem and imipenem. *Klebsiella* showed sensitivity to amikacin, gentamycin, ceftriaxone, cefepime, imipenem and Piptaz while pseudomonas showed sensitivity to cefepime, imipenem, gentamycin and Piptaz [25]. Alif YM et al., conducted a study among 12 samples and reported that *Klebsiella*, followed by *E. coli*, were the commonest organisms. The bacteria showed the highest sensitivity to cefuroxime, followed by gentamycin and ciprofloxacin [26]. A research study from North India reported a similar observation as the findings of this study showing *E. coli* as the predominant organism, followed by *Klebsiella* [27]. *E. coli* was also observed as the most common microbial isolate from the bile samples of the patients' undergoing cholecystectomy [28]. Most of the samples positive for microbial culture were found to be resistant to commonly used antibiotics such as ceftriaxone, ciprofloxacin, and amoxicillin. Based on the findings of this research, an antibiotic profiling is recommended for bile cultures to gain effective results and to prevent delays in infection control in this patient cohort.

Limitation(s)

This research study documented a pattern of organisms present in the bile of cholelithiasis patients undergoing cholecystectomy, but the samples studied were limited to a single tertiary care center. Hence, multicentric research including more patients is needed to provide better insights on antibiogram profile of the bile cultures and might be helpful in gaining better outcomes. Multicentric research might also be helpful in creating an empiric antibiotic regime applicable widely due to the inclusion of diverse patient profiles.

CONCLUSION(S)

A total of 55% of the specimens studied were found to be positive on microbial culture with a higher frequency of Gram-negative microorganisms such as *E. coli* and *Klebsiella*. Apart these *Proteus*, *Enterococcus*, *Salmonella*, and *Shigella* were also found to be positive in the study specimens. The microorganisms were found to be highly resistant to common antibiotics such as Ceftriaxone, Ciprofloxacin and Amoxicillin and found to be sensitive to meropenem, Ampicillin and Piptaz.

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