



Spectrum of Bacterial Isolates and Antibigram in Bile Contents of Patient Undergoing Cholecystectomy in A Tertiary Care Hospital of Tripura

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Abstract: *Introduction:* Diseases of the gallbladder have been recorded in humans since antiquity, with gallstones found in the mummy of princess Amenhotep of Thebes dating to 1500 BCE. Some historians believe the death of Alexander the Great may have been associated with an acute episode of cholecystitis. *Aims:* To look into the Spectrum of Bacterial Agents in Bile contents Of Patient Undergoing Cholecystectomy and Antibiotic Sensitivity Pattern. *Materials and method:* A Cross Sectional study was conducted with Prior Approval from Ethical Committee of AGMC & GBPH, AGARTALA. The study duration was January 2021 to July 2022. 187 patients were included in this study. *Result:* In our study, Bactibilia was found in 27/50 (52%) of patients. Polymicrobial flora was found in 10% of bile samples. Escherichia coli, Klebsiella pneumoniae and Enterococcus faecalis were the predominant organisms isolated. None of the anaerobes were isolated. Extended spectrum β -lactamase and AmpC β -lactamase (AmpC) production was seen in 47% and 31.5% of Enterobacteriaceae isolates respectively. Post-operative wound infection was found in six (12%) patients who had bactibilia. *Conclusion:* we concluded that, the valuable information regarding the bacteriological profile and the antimicrobial susceptibility pattern in patients undergoing cholecystectomy and admitted in AGMC and GB Pant hospital. This information can be used in formulating empirical antibiotic guidelines in patients admitted in AGMC and GB Pant hospital and planned for cholecystectomy.

Keywords: Cholecystectomy, Bile contents and Bacterial Isolates.

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INTRODUCTION

Diseases of the gallbladder have been recorded in humans since antiquity, with gallstones found in the mummy of princess Amenhotep of Thebes dating to 1500 BCE. Some historians believe the death of Alexander the Great may have been associated with an acute episode of cholecystitis. The existence of the gallbladder has been noted since the 5th century, but it is only relatively recently that the function and the diseases of the gallbladder has been documented particularly in the last two centuries (Jarnagin, 2012)

The first descriptions of gallstones appear to have been in the Renaissance, perhaps because of the low incidence of gallstones in earlier times owing to a diet with more cereals and vegetables and less meat. Anthonius Biennu's in 1506 was the first to draw a connection between symptoms and the presence of gallstones. Courvoisier, after examining a number of cases in 1890 that gave rise to the eponymous Courvoisier's law, stated that in an enlarged, non tender gallbladder, the cause of jaundice is unlikely to be gallstones. (Quick et al., 2019)

The main function of the gallbladder is to store bile, also called gall, needed for the digestion of fats in food. Produced by the liver, bile flows through small vessels into the larger hepatic ducts and ultimately through the cystic duct (parts of the biliary tree) into the gallbladder, where it is stored. At any one time, 30 to 60 ml of bile is stored within the gallbladder. When food containing fat enters the digestive tract, it stimulates the secretion of cholecystokinin (CCK) from I cells of the duodenum and jejunum. In response to cholecystokinin, the gallbladder rhythmically contracts and releases its contents into the common bile duct, eventually draining into the duodenum. The bile emulsifies fats in partly digested food, thereby assisting their absorption. Bile consists primarily of water and bile salts, and also acts as a means of eliminating bilirubin, a product of hemoglobin metabolism, from the body (Neve et al., 2003).

The bile that is secreted by the liver and stored in the gallbladder is not the same as the bile that is secreted by the gallbladder. During gallbladder storage of bile, it is concentrated 3-10 fold by removal of some water and electrolytes. This is through the active transport of sodium and chloride ions across the epithelium of the gallbladder, which creates an osmotic pressure that also causes water and other electrolytes to be reabsorbed. Bile while leaving liver composed of 97% water, bile salt 2%, bile pigments, cholesterol and fatty acid consists of 1%. Liver excrete bile at the rate of 40 ml / hr

In addition to pain, nausea, and vomiting, a person may experience a fever. If the stones block the duct and cause bilirubin to leak into the bloodstream and surrounding tissue, there may also be jaundice and itching. If this is the case, the liver enzymes are likely to be raised About 2-3% of asymptomatic patients will develop symptoms which will require cholecystectomy thus making the cholecystectomy one of the most common operation performed by general surgeons around the world. Gall bladder diseases are associated with considerable morbidity and poses great burden to the health care system around the globe. Microbial study of bile gives important information about the bacterial microflora of bile. Studies on microflora in bile revealed much information. In some studies in India has shown some correlation with gall bladder carcinoma and colonization of biliary tract with microorganism like E.coli, salmonella typhi or salmonella para typhi. In today's era of antibiotic resistance among common organism of gut flora against commonly available antibiotic poses a therapeutic challenge not only for the patients suffering from acute or chronic cholecystitis but for entire health system. It is well establish fact that antibiotic resistance is real threat for our entire generation. Therefore it becomes a necessary to study the microbial pattern of gall bladder diseases and its sensitivity to commonly available antibiotics in the markets from time to time⁴.

The majority of the isolates from infected wounds are known to be resistant to ampicillin and amoxicillin. Large numbers of S. aureus are methicillin-resistant S. aureus (MRSA) and most bacteria isolated are sensitive to quinolones, aminoglycosides and monobactam.

This study aims to look into the spectrum of bacterial agents in bile contents of patient undergoing cholecystectomy and antibiotic sensitivity pattern.

MATERIALS AND METHODS

Study Design: Cross Sectional

Study Duration: The Procedure and Data Collection was For One and Half Year (1 ½) Duration with Effect from January 2021 to July 2022

Subject Selection: Patients undergoing cholecystectomy in this hospital irrespective of sex, age, and religion with or without cholelithiasis was included in this study.

Inclusion Criteria:

Patients of cholecystitis with or without cholelithiasis, who underwent cholecystectomy was included in this study are:-

Exclusion Criteria

- Patient Not Fit For Surgery
- Patients not willing to participate in study

Ethical Committee Approval: The study was conducted with prior approval from ethical committee of AGMC & GBPH, Agartala.

Census sampling of all the patients undergoing cholecystectomy in AGMC AND GBPH department of surgery was included in this study. Informed consent taken from each patient. The aims and objectives of the study, the benefits, the confidentiality of participants and results, the voluntary nature of participation and free-will to withdraw from the study without penalty were clearly spelt out to the participants.

STUDY TOOLS

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Patients are selected as per inclusion and exclusion criteria. History is taken and examination is done as mentioned in Performa (annexure III) after taking informed consent (annexure I& II)

MATERIALS REQUIRED

Sterile cotton swab sticks, sterile disposable syringe sterile disposable gloves, normal saline solution, culture media plates like blood agar, nutrient agar, Mac Conkey agar, antibiotic discs. Biochemical tests like sugar fermentation test, citrate test, urea hydrolysis test, TSI agar, MR and indole reagents, cavities slides for motility testing.

SAMPLE COLLECTION

Bile sample was collected from patient undergoing choleccystectomy intraoperatively through 05 cc sterile syringe

MICROBIOLOGICAL PROCESSING AND TESTING OF SAMPLES

Samples cultured and Positive samples taken for further culture, staining and biochemical tests. Antibiotic sensitivity was determined by Kirby-Bauer disc diffusion technique following CLSI (UK), 2014) guidelines.

Statistical Analysis:

For statistical analysis data were entered into a Microsoft excel spreadsheet and then analyzed by SPSS 24.0. and GraphPad Prism version 5. A chi-squared test (χ^2 test) was any statistical hypothesis test wherein the sampling distribution of the test statistic is a chi-squared distribution when the null hypothesis is true. Without other qualification, 'chi-squared test' often is used as short for Pearson's chi-squared test. Unpaired proportions were compared by Chi-square test or Fischer's exact test, as appropriate. p-value ≤ 0.05 was considered for statistically significant.

RESULT AND DISCUSSION

A Cross sectional study was conducted with Prior Approval from Ethical Committee of AGMC & GBPH, AGARTALA. The study duration was January 2021 to July 2022.

In This present study, 187 bile samples were collected intra operatively from patients undergoing cholecystectomy in AGMC and GB hospital after explanation to patients prior to the operation. Samples were sent to microbiology dept for microbiological analysis and antibiotic sensitivity pattern. After the results were available the same has been analyzed.

In this study it has been observed that most of the patients belong to age group between 31- 41 age groups. Among the patient's female population 120 (64.2%) was higher than the male population 67 (35.8%) who underwent cholecystectomy.

This present study found that most of the patient belongs to Hindu community and most of the patients are from urban areas.

This study observed that most of the patients fall into lower middle socio-economic status as per revised BG Prasad criteria.

This present study demonstrated that out of the samples collected (187) there were 50 samples found to be positive for micro-organism growth. These positive samples were further analyzed for different microorganisms. Most common organism isolated from the bile was E.Coli which was 14.97%, followed by Klebsiella which was found in 8.02% samples, then salmonella which was positive in 2.14% of samples and pseudomonas was found to be positive in 1.60% samples. In the same study 3.74 % samples were found to be having positive for more than one microorganism. In this study it has been observed that 8% percent patient develop surgical site infection. Sex was not significantly related with Organism isolated in positive culture.

In similar study (Hadi et al., 2016) showed that acute cholecystitis is one of the most common acute surgical conditions. Laparoscopic cholecystectomy remains the mainstay of treatment. In patients managed non-operatively, antibiotics play an important role in the treatment of cholecystitis. The current retrospective observational study was conducted at a tertiary care hospital in Karachi, and comprised medical records of patients admitted between 2008 and 2014with acute

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cholecystitis and in whom bile cultures were obtained. Of the 509 patients with a mean age of 51.15 ± 13.4 years, early laparoscopic cholecystectomy (within 72 hours) was performed on 473 (92.9%) cases, while the rest underwent percutaneous cholecystostomy. Bile cultures were positive in 171 (33.6%) patients. Predominantly gram-negative organisms were isolated among a total of 137 (27%), with *E. coli* 63 (46%) being the most commonly isolated organism. Of the gram-positive organism, enterococcus 11 (8%) was the most common. Antibiotic sensitivities were determined. Based on their findings gram-negative coverage alone (Pushpalatha & Shoorashetty, 2012) examined that cholecystitis is a common indication for major abdominal surgeries. It may occur with or without obstruction of common bile duct. Obstruction leads to secondary bacterial infection of bile. Bactibilia is an important predisposing factor for post-operative complications. Hence, this study was designed to determine the prevalence of bacteria in bile samples of cholecystitis patients and to correlate bactibilia and post-operative wound infection. Bile samples collected intra-operatively were subjected to gram stain, culture, and antibiotic sensitivity testing. The patients were followed-up for post-operative complications. If post-operative wound infection was found, cultures were done and correlated with bacteria isolated from bile samples. Bactibilia was found in 27/50 (52%) of patients. Polymicrobial flora was found in 10% of bile samples. *Escherichia coli*, *Klebsiella pneumoniae* and *Enterococcus faecalis* were the predominant organisms isolated. None of the anaerobes were isolated. Extended spectrum β -lactamase and AmpC β -lactamase (AmpC) production was seen in 47% and 31.5% of Enterobacteriaceae isolates respectively. Post-operative wound infection was found in six (12%) patients who had bactibilia. Combination of Piperacillin-Tazobactam and Amikacin was most effective in prophylactic regimen. The organisms responsible for bactibilia were found to cause post-operative infections in the same patient warranting the use of prophylactic antibiotics in every patient undergoing cholecystectomy. All patients undergoing open cholecystectomy should receive prophylactic antibiotics to prevent post-operative wound infections.

Even in other similar study, Pushpalatha H et al (2012) examined that cholecystitis is a common indication for major abdominal surgeries. It may occur with or without obstruction of common bile duct. Obstruction leads to secondary bacterial infection of bile. Bactibilia is an important predisposing factor for post-operative complications. Hence, this study was designed to determine the prevalence of bacteria in bile samples of cholecystitis patients and to correlate bactibilia and post-operative wound infection. Bile samples collected intra-operatively were subjected to gram stain, culture, and antibiotic sensitivity testing. The patients were followed-up for post-operative complications. If post-operative wound infection was found, cultures were done and correlated with bacteria isolated from bile samples. Bactibilia was found in 27/50 (52%) of patients. Polymicrobial flora was found in 10% of bile samples. *Escherichia coli*, *Klebsiella pneumoniae* and *Enterococcus faecalis* were the predominant organisms isolated. None of the anaerobes were isolated. Extended spectrum β -lactamase and AmpC β -lactamase (AmpC) production was seen in 47% and 31.5% of Enterobacteriaceae isolates respectively. Post-operative wound infection was found in six (12%) patients who had bactibilia. Combination of Piperacillin-Tazobactam and Amikacin was most effective in prophylactic regimen. The organisms responsible for bactibilia were found to cause post-operative infections in the same patient warranting the use of prophylactic antibiotics in every patient undergoing cholecystectomy. All patients undergoing open cholecystectomy should receive prophylactic antibiotics to prevent post-operative wound infections.

In this present study positive samples were tested for antibiotic sensitivity pattern for common antibiotic available in market which are commonly being used in this part of country. Among them most sensitive antibiotics found to be IMP 26.73% followed by MEM (25.13%) DOX (22.99%), TZP (22.99%), CLI (22.99%), CRO (22.45%), CIP (21.92%), LNZ (21.40%), GEN (21.40%), CXM (20.32%), AMK (18.19%), AMC (18.19%) and CTX (16.57%).

In similar study (Suh et al., 2021) showed that biliary infections like cholecystitis and cholangitis are common and could be life threatening if treated inappropriately. Prescribing antibiotics is the key to control such infections. Occurrence of bacterial resistance to antibiotics is highly probable and should be continuously monitored. This study aimed to re-evaluate bacterial species distribution and their interaction to antibiotics in biliary infections. Total 2288 patients who were diagnosed as whether acute or chronic cholecystitis with/without concurrent cholangitis enrolled in this cohort study. All were candidate for cholecystectomy operation. In the theatre a sterile bile sample was aspirated from the gallbladder as early as the organ was exposed. Analysis was performed on culture and antibiogram results. Finally 492 (21.5%) microorganism growths were seen in all culture environments. Bacterial colonization was most common in cholangitis (63.8%) which followed by acute (26%) and chronic (10.9%) cholecystitis respectively ($p = 0.001$). *Escherichia coli*

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(58%) and Klebsiella species (12.2%) were mostly isolated pathogens. Antibigram study illustrated bacterial sensitivity of gram-negative pathogens to Imipenem (100%), Amikacin (98.1%), and Gentamicin (90.4%) which in gram-positive bacterial species was 100% to Imipenem, Vancomycin, Rifampicin, and Clindamycin. Cephalosporins as an empirical treatment for biliary infections are not suitable. Aminoglycosides including Amikacin and Gentamycin are costly beneficial as the first line for empirical antibiotic therapy in selected patients because of their good bacterial sensitivity and low expenses. Imipenem should remain for multidrug resistance species.

Present study showed that, mean Duration of hospital stay in days was significantly higher in Klebsiella positive patients compared to Escherichia coli.

Table 1: Distribution of mean OT time

	Number	Mean	SD	Minimum	Maximum	Median
OT-Time	187	60.5936	17.6030	30.0000	110.0000	60.0000

Table 2: Distribution of types of operation

Type	Frequency	Percent
Lap	18	9.62%
Open	169	90.37%
Total	187	100.0%

Table 3: Distribution of Surgical site infections and Organism

		Frequency	Percent
Surgical site infections	No	172	91.97%
	Yes	15	8.03%
	Total	187	100.0%
Organism	Escherichia coli	28	14.97%
	Klebsiella	15	8.02%
	Salmonella	4	2.14%
	Pseudomonas	3	1.60%
	Negative	137	73.26%
	Total	187	100.0%

Table 4 : Sensitivity Pattern of Antibiotics

Antibiotic	Sensitive	Resistance	Not Tested
IMP	26.73%	0.53%	72.74%
MEM	25.13%	2.13%	72.74%
DOX	22.99%	4.27%	72.74%
TZP	22.99%	4.27%	72.74%
CLI	22.99%	4.27%	72.74%
CRO	22.45%	4.81%	72.74%
CIP	21.92%	5.34%	72.74%
LNZ	21.40%	5.88%	72.74%
GEN	21.40%	5.88%	72.74%
CXM	20.32%	6.95%	72.74%
AMK	18.19%	9.09%	72.74%
AMC	18.19%	9.09%	72.74%

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CTX	16.57%	10.69%	72.74%
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CONCLUSION

In this present study most common organism isolated was it was E. coli followed by Klebsiella, Salmonella and Pseudomonas.

Most sensitive antibiotic found to be Imipenem, Meropenem Doxycycline Ciprofloxacin, Ceftriaxone and least responsive was Cefotaxime.

The present study has provided the valuable information regarding the bacteriological profile and the antimicrobial susceptibility pattern in patients undergoing cholecystectomy and admitted in AGMC and GB Pant hospital. This information can be used in formulating empirical antibiotic guidelines in patients admitted in AGMC and GB Pant hospital and planned for cholecystectomy.

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