

A Retrospective Study of Bacteriological Analysis of Bile Culture in Cholecystectomy Patients

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Abstract

Introduction: The daily excretion of bile helps to flush out microorganisms entering the biliary tract in healthy individuals. The obstruction is due to gallstones in 80% of cases. Cholecystectomy is currently a frequently performed operation. The presence of gallstones within either the gallbladder or biliary tree is associated with the bacterial colonization of the bile. **Materials and Methods:** About 5 ml bile was aspirated from all patients, this collected bile from gallbladder before cholecystectomy was transported to the laboratory in sterile test-tube. The bile samples were received in the Bacteriology laboratory in liquid medium in blood culture bottles. The bottles were incubated for 24 hrs at 37°C. The next day samples were inoculated on Blood agar and MacConkey agar and incubated overnight at 37°C aerobically. All plates were examined for visible growth. The colonies were identified as per the standard microbiological procedure. Antibiotic sensitivity testing of the microorganisms was done by modified Kirby Bauer Disc Diffusion method on Muller Hinton agar. The results were interpreted as recommended by the CLSI (Clinical Laboratory Standards Institute) Guidelines. Statistical Package for Social Science (SPSS) software, version 16, was used for data analysis. **Results:** Out of the 200 bile samples received in the Microbiology laboratory for analysis, 81 (26.47%) were culture positive. *Escherichia coli* with 30(37.03%) isolates were predominant, followed by *Pseudomonas sp.* 24(29.62%), *Klebsiella sp.* 12(14.81%), and *Citrobacter sp.* 12(11.11%). Also, 3(3.70%) isolate each of *Staphylococcus aureus* and Coagulase Negative *Staphylococcus (CONS)* was obtained. **Conclusion:** Therefore, it is very important to determine responsible pathogens and their antibiotic susceptibility pattern for the successful treatment of bile duct infections. This will help in better management in order to prevent morbidity and mortality among patients.

Keywords: Bile, CLSI, SPSS, CONS.

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Introduction

The daily excretion of bile helps to flush out microorganisms entering the biliary tract in healthy individuals. Bactibilia occurs when bile flow is impaired, and bacteria gain access to the biliary duct by the duodenal ampulla or portal circulation. Bile stasis usually occurs due to chronic obstruction. The obstruction is usually attributed to gallstones in 80% of cases. Biliary obstruction causes stasis, resulting in bacterial proliferation and dissemination.[1] eventually, the bacteria presumably translocate into the circulation, causing a systemic infection. Acute cholangitis spans a continuous clinical spectrum and can progress from local biliary infection to advanced disease with sepsis and multiple organ dysfunction syndromes.

Incidence of gallstone increases with age. It is more common in females than male (M: F = 1:4). About 50% of patients with gallstones are asymptomatic. 1–2% of asymptomatic patients will develop symptoms requiring cholecystectomy per year, making cholecystectomy one of the most common operations performed by surgeons.

Bacterial infection is the most common type of biliary tract infection, with a gram-negative preponderance. Gram-positive and anaerobic bacteria are uncommon causative agents. Viral and fungal agents are rare[3]. The typical pathogens are the Gram-negative enteric aerobes such as *Escherichia coli*, *Klebsiella* species, and *Proteus* species.

Pseudomonas aeruginosa, *Bacteroides fragilis*, and *Enterococcus faecalis* are less commonly cultured.[2] The aim of this study was to determine the Bacteriological Profile of bile culture and to study their Antibiotic Susceptibility Pattern. The resultant data will prove beneficial in identifying the causative agents of Bile infection in our hospital setting. It will be helpful in accurate antibiotic therapy, infection control protocols and prevent a prolonged hospital stay.

Materials and Methods

This was a retrospective study conducted in the Department of Microbiology, and Department of Surgery. This study was carried over a period of 1 year from Sep 2018- Sep 2019.

All the routine Bile Samples were received in the Bacteriology laboratory for culture and antibiotic sensitivity testing.

Inclusion criteria were patients undergoing cholecystectomy and patient giving informed consent for study. Patients' age >12 years and <65 years, proven cases of gallstone disease for cholecystectomy both open and laparoscopic cholecystectomy.

Exclusive criteria: Acute cholecystitis, acute a calculus cholecystitis emphysema gallbladder mucocele of the gallbladder, jaundice patients, and gallstones with multiple common bile duct stones (multiple CBD and intrahepatic stones). Patients were refused surgery.

In case of laparoscopic cholecystectomy, bile was collected from the excised gallbladder. The sample was collected in sterile bottle and was transferred to microbiology laboratory. The bile samples were received in the Bacteriology laboratory in liquid medium in blood culture bottles. The bottles were incubated for 24 hrs at 37°C. The

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next day samples were inoculated on Blood agar and MacConkey agar and incubated overnight at 37°C aerobically. All plates were examined for visible growth. The colonies were identified as per the standard microbiological procedure. Antibiotic sensitivity testing of the microorganisms was done by modified Kirby Bauer Disc Diffusion method on Muller Hinton agar, and the results were interpreted as was recommended by the CLSI (Clinical Laboratory Standards Institute) Guidelines. Statistical Package for Social Science (SPSS) software, version 22, was used for data analysis[3,4]

Results

Out of the 200 bile samples received in the Microbiology laboratory for analysis, 81 (26.47%) were culture positive. *Escherichia coli* with 30(37.03%) isolates were predominant, followed by *Pseudomonas sp.* 24(29.62%), *Klebsiella sp.* 12(14.81%), and *Citrobacter sp.* 12(11.11%). Also, 3(3.70%) isolate each of *Staphylococcus aureus* and *CONS (S.epidermidis and S.hemolyticus)* was obtained [Table 1].

Table 1:Percentage distribution of isolates

S.No	Organism	No.of Isolates
1	<i>Escherichia coli</i>	30(37.03%)
2	<i>Pseudomonas spp.</i>	24(29.62%)
3	<i>Klebsiella spp.</i>	12(14.81%)
4	<i>Citrobacter spp.</i>	9(11.11%)
5	<i>Staphylococcus aureus</i>	3(3.70%)
6	CONS	3(3.70%)
7	Total	81(100%)

Table 2: Antibiotic Sensitivity Profile of Gram-Negative Isolates (N=25)

S.No		E.Coli (N=30)	<i>Pseudomonas sp.</i> (N=24)	<i>Klebsiella sp.</i> (N=12)	<i>Citrobacter sp.</i> (N=9)
1	AMP	3 (10%)	3 (12.5%)	0 (0%)	0 (0%)
2	AMC	3 (10%)	0 (0%)	0 (0%)	0 (0%)
3	PIT	18 (60%)	18 (75%)	6 (50%)	6 (66.6%)
4	CPM	21 (70%)	15 (62.5%)	9 (75%)	6 (66.6%)
5	CXM	9 (30%)	18 (75%)	3 (25%)	3 (33.3%)
6	CTR	18 (60%)	15 (62.5%)	9 (75%)	6 (66.6%)
7	AO	24 (80%)	18 (75%)	9 (75%)	6 (66.6%)
8	MR	27 (90%)	21 (87.5%)	9 (75%)	9 (100%)
9	AK	24 (80%)	16 (75%)	9 (75%)	6 (66.6%)
10	GEN	15 (50%)	15 (62.5%)	6 (50%)	6 (66.6%)
11	TOB	27 (90%)	21 (87.5%)	9 (75%)	9 (100%)
12	CIP	12 (40%)	12 (50%)	6 (50%)	3 (33.3%)
13	COT	9 (30%)	9 (37.5%)	6 (50%)	3 (33.3%)
14	CEF-SUL	-	24 (100%)	-	-
15	CL	-	21 (87.5%)	-	-
16	PB	-	21 (87.5%)	-	-
17	CPM-TZ	-	21 (87.5%)	-	-

AMP=Ampicillin, AMC=Amoxicillin-clavulanic acid, PIT= Piperacillin-Tazobactam, CPM=Cefepime, CXM= Cefuroxime, CTR= Ceftriaxone, AO= Aztreonam, MR= Meropenem, AK= Amikacin, GEN=Gentamicin, TOB=Tobramycin, CIP=Ciprofloxacin, COT=Co-trimaxazole, CEF-SUL= Cefoperazone-sulbactam, CL=Colistin, PB=Polymyxin-B, CPM-TZ= Cefepime-tazobactam

Among the gram-positive isolates, Vancomycin and Linezolid were the most effective, showing 100% sensitivity. The other antibiotics which showed good sensitivity were Gentamicin, Cefoxitin, and Clindamycin.

Among the gram-negative isolates :-Meropenem and Tobramycin where the most effective with

E.coli showing – 90% sensitivity
Pseudomonas showing – 87.5% sensitivity
Klebsiella showing – 75% sensitivity
Citrobacter showing – 100% sensitivity

Table 3:Distribution of Surgical Treatment

Procedure	Number of Cases
Open cholecystectomy	121
Lap cholecystectomy	74
Lap converted to open	5

As per table 3 one twenty-one patients underwent open cholecystectomy, 74 patients were undergoing laparoscopy cholecystectomy and 5 cases laparoscopy converted to open Table 5.

Discussion

In the present study, 200 bile samples were analyzed for the presence of microorganisms, out of which 81 (26.47%) showed bacterial growth, which was in accordance with Ahmed M et al[1] (23.6%) while studies conducted by Hadi YB et al and Capoor MR et al[3] found a higher rate of bacterial growth, i.e.(33.6%) and (32%) respectively.

Normally bile is sterile because of various anatomical and physiological mechanisms[4]. A competent sphincter of Oddi prevents intestinal contents from refluxing into the bile duct, and antegrade bile flow periodically flushes the biliary system, keeping it free of organisms. In addition, bile components, including bile salts and immunoglobulin A (IgA), have antibacterial characteristics protecting the biliary tree from bacteria.[5] Prolonged bile duct obstruction leads to impaired intestinal wall barrier function. Consequently, bacteria can colonize bile easier. Thus *E. coli* is the most common bacterium resulting in the development of bile infection. The enteric gram-negative aerobes are the commonly found organisms in infected bile. In the present study, *Escherichia*

coli (37.03%) was the most common organism isolated from bile cultures. Other organisms were *Pseudomonas sp.* (29.62%), *Klebsiella sp.* (14.81%), and *Citrobacter sp.* (11.11%). Similar results were seen in Grizas S et al [6] and Parekh PM et al.

Studying the demographic profile in the present study, the maximum number of patients were female (73.52%) and in age-group 36-40 years (24.50%) as observed by Hassan SM et al [7] and Kumar M et al. [8]

Antibiotic sensitivity pattern of Gram-positive cocci showed good sensitivity against Vancomycin and Linezolid, which was also found in a study by Sharma V et al. [9,10,11]

Antibiotic sensitivity pattern of Gram-negative organisms showed good sensitivity against Meropenem and Tobramycin

Conclusion

This study concludes that apart from surgery, prompt administration of appropriate antibiotics to control the biliary tract infection is also very important. Despite this, there are no published guidelines for antibiotic treatment in bile duct infections. Therefore, it is very important to determine responsible pathogens and their antibiotic sensitivity pattern for the successful treatment of bile duct infections. This will help in better management in order to prevent morbidity and mortality among patients.

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