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Original Research Article

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

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Abstract

Introduction: In healthy people, the regular excretion of bile aids in flushing out bacteria that enter the biliary tract. Gallstones are the cause of the obstruction in 80% of cases. Currently, cholecystectomy procedures are commonly carried out. The bacterial colonization of the bile is related to the occurrence of gallstones in the gallbladder or biliary tree.

Materials and Methods: Each patient had approximately 5 ml of bile aspirated from their gallbladder before having a cholecystectomy, which was then delivered to the lab in a sterile test tube. The Bacteriology laboratory received the bile samples in liquid media in blood culture bottles. At 37°C, the bottles were incubated for 24 hours. Samples were placed on Blood and MacConkey agar the following day and incubated aerobically at 37°C overnight. Every plate was checked for evidence of growth. In accordance with the accepted microbiological practice, the colonies were identified. The modified Kirby Bauer Disc Diffusion method was used to examine the bacteria' antibiotic sensitivity on Muller Hinton agar.

Results: 81 (26.47%) of the 200 bile samples that were submitted to the microbiology lab for investigation were culture-positive. Escherichia coli 30 (37.03%), Pseudomonas sp. 24 (29.62%), Klebsiella sp. 12 (14.81%), and Citrobacter sp. 12 (11.11%) were the most common isolates. Additionally, 3 (3.7%) isolates of each Coagulase Negative Staphylococcus (CONS) and Staphylococcus aureus were found.

Conclusion: To effectively treat bile duct infections, it is crucial to identify the microorganisms at fault as well as their pattern of drug susceptibility. Better management will result from this, lowering patient morbidity and mortality rates.

Keywords: Bile, CLSI, SPSS, CONS.

Introduction

In healthy people, the regular excretion of bile aids in flushing out bacteria that enter the biliary tract. When bile flow is compromised, bacilli enter the biliary channel through the duodenal ampulla or portal circulation. Bile stasis typically results from a persistent blockage. Gallstones are typically to blame for the

obstruction in 80% of situations. Stasis brought on by biliary obstruction promotes bacterial growth and spread. Eventually, the germs apparently translocate into the circulation and result in a systemic infection. Acute cholangitis has a wide range of clinical manifestations that can range from a localized biliary infection to a severe condition that includes sepsis and numerous organ dysfunction syndromes.[1] With a predominance of gram-negative bacteria, bacterial infection is the most prevalent kind of biliary tract infection. Gram-positive bacteria and anaerobic bacteria are rare culprits. The most common pathogens are Gram-negative enteric aerobes such Escherichia coli, Klebsiella species, and Proteus species. Viral and fungal agents are uncommon. Less often cultured bacteria include Pseudomonas aeruginosa, Bacteroides fragilis, and Enterococcus faecalis.[2] The goal of this work was to identify the bacteria in bile culture and analyze their antibiotic susceptibility pattern. The resulting information will be useful in determining the agents responsible for bile infection in our hospital context. Accurate antibiotic medication, infection control procedures, and a short hospital stay will all benefit from it.

Materials and Methods

This was a retrospective study conducted in the Department of Pathology and Department of Surgery. This study was carried over a period of 1 year from Sep 2021- Sep 2022. The routine Bile Samples were received in the Central Pathology laboratory for 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.

Bile was extracted from the removed gallbladder in a laparoscopic cholecystectomy. The sample was gathered in a clean vial and delivered to the microbiology lab. The laboratory received the bile samples in liquid media in blood culture bottles. At 37 °C, the bottles were incubated for 24 hours. Samples were placed on Blood and MacConkey agar the following day and incubated aerobically at 37°C overnight. Every plate was checked for evidence of growth. In accordance with the accepted microbiological practice, the colonies were identified. The microorganisms were subjected to modified Kirby Bauer Disc Diffusion antibiotic sensitivity testing on Muller Hinton agar, and the findings were interpreted in accordance with CLSI (Clinical Laboratory Standards Institute) Guidelines. Statistical Package for Social Science (SPSS) software, version 22, was used for data analysis.

Results

Table 1: Percentage distribution of isolates

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

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

S. No.		E.Coli (N=30)	Pseudomonas sp. (N=24)	Klebsiella sp. (N=12)	Citrobacter sp. (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. [4] and Capoor MR et al. [3] found a higher rate of bacterial growth, i.e.(33.6%) and (32%) respectively.

Due to multiple anatomical and physiological processes, bile is typically sterile. An effective sphincter of Oddi prevents intestinal contents from flowing backward into the bile duct, and periodic antegrade bile flow cleanses the biliary system, keeping it clean and free of pathogens. Additionally, the biliary tree is shielded against microorganisms by the antibacterial properties of bile components such bile salts and immunoglobulin A (IgA). 5 Long-term bile duct obstruction compromises the barrier function of the intestinal wall. As a result, bile can become more colonized by bacteria. Therefore, the most frequent bacterium causing the development of bile infection is E. coli. The most frequent organisms discovered in bile that has been infected are enteric gram-negative aerobes. Escherichia coli (37.03%) was the most frequent bacterium identified from bile cultures in the current investigation. 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.[2]

According to Hassan SM et al. [7] and Kumar M et al., who studied the demographic profile in the current study, the majority of patients were female (73.52%) and in the age range 36–40 years (24.50%). [8] As reported in a study by Sharma V et al., the antibiotic sensitivity pattern of Gram-positive cocci showed good sensitivity against [9]Vancomycin and Linezolid. Gram-negative bacteria's antibiotic sensitivity pattern revealed good sensitivity to [10]Meropenem and Tobramycin.[11]

Conclusion

This study's findings suggest that, in addition to surgery, timely use of effective antibiotics to treat biliary tract infections is equally crucial. In spite of this, there are no published recommendations for the administration of antibiotics for bile duct infections. To effectively treat bile duct infections, it is crucial to identify the microorganisms at fault as well as their pattern of antibiotic susceptibility. Better management will result from this, lowering patient morbidity and mortality rates.

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