

Original Research Article

Bacteriological Analysis of Bile Culture from a Tertiary Care Hospital

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ABSTRACT

Background: Human bile though sterile normally, can become infected in Biliary tract obstruction due to entry of microorganisms through various routes like Papilla of Vater or hematogenous, leading to bactobilia.

Aim and Objectives: To determine the Bacteriological Profile and Antibiotic Susceptibility Pattern of Bile cultures from a Tertiary Care Hospital.

Setting and Design: A retrospective study was conducted in the Dept. of Microbiology, GMC, Jammu for a period of 1 year i.e. Sep 2016-2017.

Methods: The bile samples were collected in Blood Culture Bottles and incubated for 24 hrs. After that the samples were inoculated on Blood agar and MacConkey agar and incubated overnight at 37°C. The isolates were identified by standard microbiological methods. Antibiotic Susceptibility Testing was done by Kirby-Baur Disc Diffusion Method as per CLSI guidelines.

Results: 27 (26.47%) patients showed bile culture positive. The most commonly isolated gram negative organisms were Escherichia coli, Pseudomonas spp and Klebsiella spp. Of the gram positive isolates, Staphylococcus aureus and CONS were obtained. Maximum number of patients were females and in age group 35-40 years. Studying the sensitivity profile, the gram negative isolates were most sensitive to Meropenem, Amikacin and Tobramycin while the gram positive isolates were more sensitive to Linezolid and Vancomycin.

Conclusion: This study concludes that a bile sample for microbiological analysis becomes a valuable diagnostic tool as it leads to more accurate selection of antibiotics for the proper management.

Keywords: Bactobilia, CLSI, Microbiological analysis.

INTRODUCTION

Bile is normally sterile. The daily excretion of bile helps to flush out microorganisms entering the biliary tract in healthy individuals. Bactobilia occurs when bile flow is impaired and bacteria gain access to the biliary duct by the papilla 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 an increase in ductal pressure, 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 a local biliary infection to advanced disease with sepsis and multiple organ dysfunction syndrome. [2]

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, while *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 prolonged hospital stay.

MATERIALS AND METHODS

STUDY DESIGN- This was a retrospective study conducted in the Department of Microbiology, Government Medical College, Jammu. This study was carried over a period of 1 year from Sep 2016- Sep 2017.

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

SAMPLE PROCESSING – The bile samples were received in the Bacteriology laboratory in liquid medium in blood culture bottles. The bottles were incubated for 24 hrs at 37C. Next day samples were inoculated on Blood agar and MacConkey agar and incubated overnight at 37⁰C aerobically. All plates were examined for visible growth. The colonies were identified as per 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 ANALYSIS- Statistical Package for Social Science (SPSS) Software, version 16 was used for data analysis.

RESULTS

Out of the 102 bile samples received in the Microbiology laboratory for analysis, 27 (26.47%) were culture positive.

Escherichia coli with 10(37.03%) isolates was predominant followed by *Pseudomonas sp.* 8(29.62%), *Klebsiella sp.* 4(14.81%) and *Citrobacter sp.* 3(11.11%). Also 1(3.70%) isolate each of *Staphylococcus aureus* and *CONS* was obtained [Table 1].

Table- 1: Percentage distribution of isolates.

| Organism | No. of isolates (%) |
|------------------------------|---------------------|
| <i>Escherichia coli</i> | 10(37.03%) |
| <i>Pseudomonas spp.</i> | 8(29.62%) |
| <i>Klebsiella spp.</i> | 4(14.81%) |
| <i>Citrobacter spp.</i> | 3(11.11%) |
| <i>Staphylococcus aureus</i> | 1(3.70%) |
| <i>CONS</i> | 1(3.70%) |
| TOTAL | 27(100%) |

Of the 27 culture positive cases, 8(29.62%) were of acute cholecystitis and 19(70.37%) were of chronic cholecystitis.

This study included 102 patients, 75(73.52%) were female and 27(26.47%) were male. Maximum number of patients 25(24.50%) were in age-group 36-40 years followed by 16(15.68%) in age group 61-65 years.

As far as antibiogram of bacteria is concerned, gram negative isolates showed highest sensitivity to Meropenem, Tobramycin and Amikacin. *Escherichia coli* showed 90% sensitivity to both Meropenam and Tobramycin. The other antibiotics that were found effective were Aztreonam, Cefepime and Piperacillin- tazobactam. The isolates were least sensitive to Cotrimoxazole, Ampicillin and Amoxicillin-clavulanic acid. *Pseudomonas sp.*, *Klebsiella sp.*, and *Citrobacter sp.* showed 100% resistance against Amoxicillin-clavulanic acid

In the treatment of *Pseudomonas sp.* Cefepime-Tazobactam (100%), Cefoperazone-sulbactam (87.5%), Colistin (87.5%) and Polymyxin B (87.5%) were highly effective [Table 2].

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

| | E.coli (N=10) | Pseudomonas sp. (N=8) | Klebsiella sp. (N=4) | Citrobacter sp. (N=3) |
|---------|------------------|--------------------------|-------------------------|--------------------------|
| AMP | 1(10%) | 1(12.5%) | 0(0%) | 0(0%) |
| AMC | 1(10%) | 0(0%) | 0(0%) | 0(0%) |
| PIT | 6(60%) | 6(75%) | 2(50%) | 2(66.6%) |
| CPM | 7(70%) | 5(62.5%) | 3(75%) | 2(66.6%) |
| CXM | 3(30%) | 3(37.5%) | 1(25%) | 1(33.3%) |
| CTR | 6(60%) | 5(62.5%) | 3(75%) | 2(66.6%) |
| AO | 8(80%) | 6(75%) | 3(75%) | 2(66.6%) |
| MR | 9(90%) | 7(87.5%) | 3(75%) | 3(100%) |
| AK | 8(80%) | 6(75%) | 3(75%) | 2(66.6%) |
| GEN | 5(50%) | 5(62.5%) | 2(50%) | 2(66.6%) |
| TOB | 9(90%) | 7(87.5%) | 3(75%) | 3(100%) |
| CIP | 4(40%) | 4(50%) | 2(50%) | 1(33.3%) |
| COT | 3(30%) | 3(37.5%) | 2(50%) | 1(33.3%) |
| CEF-SUL | - | 8(100%) | - | - |
| CL | - | 7(87.5%) | - | - |
| PB | - | 7(87.5%) | - | - |
| CPM-TZ | - | 7(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=Cotrimaxazole, 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.

DISCUSSION

In the present study, 102 bile samples were analyzed for the presence of microorganisms out of which 27 (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 higher rate of bacterial growth i.e.(33.6%) and (32%) respectively.

Normally bile is sterile because of various anatomical and physiological mechanisms. A competent sphincter of Oddi prevents intestinal contents from refluxing into the bile duct, and anterograde 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 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. [2]

Studying the demographic profile in the present study, 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 of Gram negative isolates revealed maximum sensitivity to Meropenem. Similar results were seen in Ahmed M et al [1] and Suna et al. [5] This shows that carbapenems are extremely effective against Gram negative bacterial infection. Good sensitivity was seen against Amikacin, Tobramycin which reveals that Aminoglycosides are a good option for treatment of Gram negative infection but due to their excessive use, resistance against them is also showing a rising curve. Resistance to second generation Cephalosporins has increased while third and fourth generation Cephalosporins show a good promise against gram negative organisms. Similar findings were seen in Parekh MP et al. [2] The isolates were least sensitive to

Amoxicillin-clavulanic acid and Ampicillin. High rate of resistance shows that Penicillins are no longer an effective treatment option. Cefepime-Tazobactam, Cefoperazone-sulbactam, Colistin and Polymyxin B were highly effective in treatment of Pseudomonas infection.

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

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 susceptibility pattern for 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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