MICROBIOLOGICAL PROFILE OF BILE IN CHOLELITHIASIS AND THEIR IMPLICATION IN CAUSING POST OPERATIVE WOUND INFECTIONS
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ABSTRACT: BACKGROUND: 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.

MATERIALS AND METHODS: 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.

RESULTS: Bactibilia was found in 43/100 (43%) of patients. Polymicrobial flora was found in 7% of bile samples. Escherichia coli, citrobacter, Klebsiella pneumoniae and pseudomonas were the predominant organisms isolated. Post-operative wound infection was found in ten (10%) patients who had bactibilia. Amikacin, gentamicin, ceftriaxone sulbatum, pipracillin tazobactum, imipenem were among most effective in prophylactic regimen.

CONCLUSION: 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 cholecystectomy should receive prophylactic antibiotic to prevent post-operative wound infections.

KEYWORDS: Cholelithiasis, cholecystitis, bile, bactibilia, wound infection.

INTRODUCTION: Gallstones are complex bio mineralized deposits formed in the gallbladder. Gallstone disease is still a major health problem throughout the world. 20–30% of western and around 10% of non-western population is affected by gallstones.[¹,²]

They are generally classified into three major types: pure cholesterol, pigment and mixed gallstone. Pigment gallstones are further subdivided into laminated brown stones and amorphous black stones.[³]

Different factors have been implicated in the causation of gallstones, amongst which contamination of the bile is an important factor. According to Moynihan aphorism “Gall stones are the tombs erected in the memory of the bacteria”.

Etiologic role of aerobic and anaerobic bacteria in gallbladder disease has been proposed but their pathogenic role is not well established.

Infected bile is seen in 30% of patients with cholelithiasis. The biliary infection is caused by various types of organisms ranging from aerobic gram positive to gram negative to anaerobic organisms. Aerobic organisms cause 94% of biliary tract infections while anaerobic organisms cause the rest. Most common aerobic organisms associated with cholecystitis are Escherichia coli, Klebsiella species and Enterococcus faecalis, which constitute the normal intestinal flora. Bacteria are
commonly found in inflamed gallbladder and in patients with cholelithiasis, whereas evidence suggests that normal bile is sterile.(4)

Bactibilia is an important predisposing factor in post-operative septic complications like gram negative septicemia, post-operative wound infections, post-operative cholangitis, respiratory and urinary tract infections.[5]

Studies have found post-operative wound infections following cholecystectomy to be around 2-3%.[5]

This study was undertaken to determine the frequency of microbial contamination of bile (bactibilia), its antibiotic sensitivity pattern and its correlation with post-operative wound infection.

**MATERIAL AND METHOD:** The study was carried out in 100 consecutive cases of cholelithiasis who underwent cholecystectomy (laparoscopic or open) in a tertiary care hospital. Data on microbial profile of bile and post-operative outcome along with histopathology was analyzed.

All the cases of symptomatic cholelithiasis operated by open or laparoscopic cholecystectomy were included in the study. Patients who were not willing or lost in follow-up and who are below 16 year of age were excluded from the study. A detailed history and clinical examination was done. All routine blood investigations including complete blood count, s. urea, s. creatinine, s. electrolytes, liver function test, blood sugar, chest X-ray and ECG were done in selected patients. Ultrasound was used as diagnostic tool for gall stone disease.

Selection of the patients for the procedure was on clinical ground. Patients undergoing open and laparoscopic cholecystectomy were assessed by the anaesthetist a day prior to surgery. Consent was taken from the patient after explaining the nature of procedure and potential for conversion from laparoscopic approach to open cholecystectomy. After opening the abdomen and confirming the diagnosis, bile was aspirated from the gallbladder at fundus in a five milliliter syringe. In laparoscopic cases, bile was collected by using long spinal needles through the abdominal wall under direct vision through the telescope. All these specimens were sent immediately to microbiology laboratory for gram’s staining & culture and sensitivity examination. The specimens were cultured aerobically.

Extracted gall stones dried & sent for chemical examination to ascertain its type. Patients who develop post-operative wound infections were further investigated by doing appropriate cultures and other investigations.

**RESULTS:** A total of 100 patients with cholecystitis undergoing surgery were studied. 79 were females and 21 were males (ratio 3.76:1). The mean age was 44 ± 17 years. 91 cases done by laparoscopic cholecystectomy while remaining done by open. Cholesterol stone found in 19, mixed in 73, pigmented in 8 cases. Acute cholecystitis found in 14 cases, malignancy in 3 while remaining cases were chronic cholecystitis.

Aerobic culture of bile yielded growth in 43 cases. Bactibilia was found in 31/83 patients of chronic cholecystitis, 10/14 of acute, 2/3 neoplasms. Polymicrobial flora was seen in seven samples, rest were monomicrobial. E. coli, (15) citrobacter (10) Klebsiella pneumonieae, (9) pseudomonas (6) and E. faecalis (3) were the predominant organisms isolated. (Table. 1)
TABLE 1: Organism isolated from bile samples

| Organism      | No. of cultures | %     |
|---------------|-----------------|-------|
| E. coli       | 15              | 34.88 |
| Klebciella    | 9               | 20.93 |
| Pseudomonas   | 6               | 13.95 |
| Citrobacter   | 10              | 23.25 |
| Enterococcus  | 3               | 6.98  |
| Others        | 6               | 13.95 |
| **Total**     | **43**          | **100**|

TABLE 02: Mixed flora isolated from bile samples

| Mixed flora                              | Total no. | %     |
|------------------------------------------|-----------|-------|
| E Coli + Citrobacter                     | 2         | 4.65  |
| E Coli + Pseudomonas                     | 2         | 4.65  |
| E Coli + Klebciella                      | 1         | 2.32  |
| E Coli + Proteus                         | 1         | 2.32  |
| Pseudomonas + Acinetobacter              | 1         | 2.32  |
| **Total**                                | **7**     | **16.28**|

E. coli and K. pneumoniae showed high level of resistance to third generation cephalosporins. Both the organisms were sensitive to piperacillin-tazobactam and Amikacin. Pseudomonas aeruginosa showed 100 % sensitivity to amikacin and imipenem. Salmonella was 100% sensitive to levofloxacin and ceftriaxone sulbactum.

Among the gram positive Organisms methicillin resistant Staphylococcus aureus and streptococcus were 100 % sensitive to vancomycin and linezolid. (Table 3)

| Drugs                          | Ecoli% | Klebciella% | Citrobacter% | Pseudomonas% | Enterobacter% | MRSA% | Streptococcus% | Salmonella% |
|-------------------------------|--------|-------------|--------------|--------------|---------------|-------|----------------|-------------|
| Ampicillin                    | -      | -           | -            | -            | 33.3          | -     | -              | -           |
| Co-amoxiclav                  | -      | -           | 20           | -            | 66.6          | -     | -              | -           |
| Pipracillin-tazobactum        | 76.9   | 66.6        | 70           | 83.3         | -             | -     | -              | 50          |
| Ceftriaxone                   | 26.6   | 33.3        | 50           | 33.3         | -             | -     | -              | 50          |
| Ceftriaxone sulbactum         | 80     | 55.5        | 70           | 50           | -             | -     | 100            | 100         |
| Amikacin                      | 86.6   | 66.6        | 70           | 100          | -             | -     | -              | 50          |
| Gentamicin                    | 80     | 44.4        | 50           | 50           | -             | -     | -              | 50          |
| Imipenem                      | 93.3   | 55.5        | 80           | 100          | -             | -     | -              | 50          |
| Cotrimox-azole                | 46.6   | 33.3        | 30           | -            | 66.6          | 100   | 50             | -           |
| Ciproflo-xacin                | 66.6   | 22.2        | 40           | 50           | -             | -     | -              | 50          |
| Levo-floxacin                 | 66.6   | 33.3        | -            | -            | -             | -     | -              | 100         |
| Linezolid                     | -      | -           | -            | 100          | 100           | 100   | 100            | -           |
| Van-comycin                   | -      | -           | -            | -            | 100           | 100   | 100            | -           |

TABLE 03: Antibiotic sensitivity pattern of organisms isolated from bile
Post-operative wound infection was found in 10 patients who had bactibilia (epigastric port in laparoscopic cases). Pus samples from all the 10 cases grew organisms which were similar to the organisms in the bile cultures.

4 cases had post-operative infection with E. Coli. 2 cases with P. aeruginosa. 2 case had post-operative infection with E. coli and citrobacter species. 1 case had post-operative infection with E. coli and Klebsiella species. 1 case was infected by Methicillin Resistant Staphylococcus aureus (MRSA).

All the patients with sterile bile cultures did not suffer from post-operative wound infection.

DISCUSSION: Bile in individuals with normal biliary tract is sterile. Presence of biliary obstruction in few cases of cholelithiasis, ascending infection from duodenum or bacterial translocation from portal vein leads to bacterial colonization in biliary system. In this series, the positive bile culture in cases of cholelithiasis was found in 43%, which is considerably higher than that reported by Sabir (16%) and Van Leeuwen (16.4%) but coincides with Csendes (46%) and Guo (66.7%).

In the present study the most common organisms isolated from bile samples were E. coli, Citrobacter, K. pneumoniae, pseudomonas and E. faecalis as has been reported in other studies. Post-operative wound infection was found in 10% of patients who had bactibilia. Patients with sterile bile cultures did not develop any post-operative infections. Isolates obtained from post-operative wound samples were identical (biochemical reactions and anti-biogram) with those obtained from bile samples suggesting endogenous origin of infection. So the study recommends bile cultures in all patients undergoing cholecystectomy.

The present study shows that piperacillin-tazobactam and amikacin are highly effective against aerobic organisms. When these antibiotics are used in combination they can cover both gram positive and gram negative organisms. Prophylactic antibiotics should be continued 5-7 days post-operatively. Choudhary et al11 in their study showed that the use of prophylactic antibiotics neither prevented infection nor decreased hospital stay. However, Chandrashekhar et al12 in a prospective study found a lower rate (3%) of post-operative wound infections among the patients who received prophylactic antibiotics (23%).

The increased incidence of post-operative infection among patients having bactibilia warrants the use of prophylactic antibiotics. The empirical antibiotics should cover both gram positive and gram negative organisms. Although, cephalosporins are considered to be effective for prophylaxis in biliary disease, raising incidence of resistance warrants use of alternative antibiotics.

CONCLUSION: Aerobic organisms are most commonly isolated from bile samples. 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. Increased incidence of resistance among the isolates of bactibilia demands appropriate use of antibiotics in clinical practice.

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