Bacteriological Profile of Bile and Antibiogram in Cholecystectomy Patients

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Abstract
Cholelithiasis is one of the most common surgical condition affecting gastrointestinal tract in North India and cholecystectomy is one of the most common surgeries done.
Aims and Objectives: This study was done to evaluate the microbiological profile of bile from gall bladder in patients undergoing cholecystectomy and to determine the appropriate antibiotic for preoperative prophylaxis in cholecystectomy patients based on the microbiological profile of bile.
Material and Methods: The study was conducted in 100 patients who had undergone cholecystectomy in our unit in the department of surgery over a period of one year from July 2014 to June 2015. The samples were processed in the Department of Microbiology.
Results: 22% of bile samples were found positive for microorganisms. E.coli was found to be the commonest organism found in 64% of positive samples.
Conclusion: Bile is generally sterile but might be infected in cholelithiasis. Incidence of bacterbilia varies from 10% to 70%.

Introduction
Cholelithiasis is one of the most common surgical condition affecting gastrointestinal tract in North India and cholecystectomy is one of the most common surgeries done. (1) Human bile is normally sterile. However in biliary tract obstruction bacteria may gain access to the biliary tract through papilla of vater or portal circulation. Systemic bacteremia may occur in severe cases. In 85 -95 % of cases inflammation of gall bladder is associated with calculi. (2) Whether the stones represent a cause or effect has not been established. Persistent infection of biliary tract is one of the causes of gall stone formation.

Aims and Objectives
This study was done to evaluate the microbiological profile of bile from gall bladder in
patients undergoing cholecystectomy and to determine the appropriate antibiotic for preoperative prophylaxis in cholecystectomy patients based on the microbiological profile of bile.

**Material and Methods**

The study was conducted in 100 patients who had undergone cholecystectomy in our unit in the department of surgery over a period of one year from July 2014 to June 2015. The samples were processed in the Department of Microbiology. Inclusion criteria had all patients of choledolithiasis who underwent cholecystectomy including complicated cases having biliary colic, acute cholecystitis, chronic cholecystitis, empyema of gall bladder, mucocele, perforation of gall bladder, biliary obstruction, acute cholangitis or acute pancreatitis. Any immunocompromised patient/organ transplant recipient, patients who had underwent any intervention eg ERCP and patients who had already been given antibiotics were excluded.

Routine pre-operative investigations were done. No prophylactic antibiotics were given prior to surgery. Operative findings were recorded like size and colour of gall bladder, size and number of stones, presence of mucocele or empyema, colour of bile, diameter of common bile duct(CBD) and presence of stone in it. During surgery 5-10 ml of bile sample was aspirated with sterile syringe from gall bladder immediately after cholecystectomy. This was transferred within one hour of surgery in brain heart infusion broth to the microbiology lab. The sample was subcultured on blood agar and MacConkey agar plates and incubated at 37 degree Celsius for 24 hours. The growth obtained was identified on the basis of colony morphology and standard biochemical tests. Antibiotic susceptibility was determined as per Clinical Laboratory Standards Institute (CLSI) guidelines. All the data collected was analysed to find out the bacteriological profile in bile of patients and the antibiotic profile of the isolated organisms.

**Results**

Age of the patients ranged from 17 to 76 yrs with mean age of 46.2 yrs. Maximum patients (33%) were in 41-50 yrs age group. Male to female ratio was 1:4.2. 15% patients had associated comorbidities in the form of hypertension, diabetes mellitus, tuberculosis, bronchial asthma or heart disease.

Most of the routine haematological investigations were normal in all patients except 4 patients who had a total leucocyte count of more than 12000/mm3. Ultrasonography revealed contracted gall bladder in 10 patients, mucocele in 8 patients and empyema in 2 patients. Others had distended gall bladder. Multiple stones were present in 80 of these patients. CBD was dilated with stones in 15 patients and without stones in 8 patients. Features suggestive of acute cholecystitis was present in 5 patients and wall echo sign in 5 patients.

22% of bile samples were found positive for microorganisms. Results of samples are shown in Table 1. Antibiotic susceptibility of the bacteria is shown in Table 2.

Table 1: Type of Bacteria Grown in the positive samples

| Bacteria             | Total number | Percentage |
|----------------------|--------------|------------|
| Escherichia coli     | 14           | 64%        |
| Enterococcus sps     | 3            | 13.5%      |
| Enterobacter sps     | 1            | 4.5%       |
| Staph aureus         | 1            | 4.5%       |
| Salmonella sps       | 1            | 4.5%       |
| Pseudomonas          | 1            | 4.5%       |
| Citrobacter freundii | 1            | 4.5%       |
Table 2: Antibiogram of the bacteria isolated

|                | E.coli | Enterococcus | Enterobacter | Staph aureus | Salmonella | Pseudomonas | Citrobacter freundii |
|----------------|--------|--------------|--------------|--------------|------------|-------------|---------------------|
| Ampicillin     | 83%    | 100%         | -            | -            | 0%         | -           | -                   |
| Amoxyclav      | 30%    | -            | 100%         | -            | -          | -           | -                   |
| Cefazidine     | 50%    | -            | 100%         | -            | -          | 100%        | -                   |
| Ciprofloxacin  | 70%    | -            | 100%         | -            | -          | 100%        | -                   |
| Cefepime       | 100%   | -            | -            | -            | -          | -           | -                   |
| Cotrimoxazole  | 100%   | -            | -            | -            | -          | -           | -                   |
| Ceftriaxone    | 100%   | -            | -            | 100%         | -          | -           | -                   |
| Piperacillin   | -      | -            | -            | -            | -          | -           | -                   |
| Amikacin       | 30%    | -            | -            | -            | -          | 100%        | -                   |
| Gentamicin     | 100%   | 100%         | 100%         | 100%         | -          | 0%          | -                   |
| Tobramycin     | 100%   | -            | -            | -            | -          | -           | -                   |
| Streptomycin   | -      | 100%         | -            | -            | -          | -           | -                   |
| Imipenem       | 100%   | 33%          | 100%         | -            | -          | -           | 100%                |
| Meropenem      | 50%    | -            | -            | -            | -          | -           | -                   |
| Tetracycline   | 0%     | 100%         | -            | -            | -          | -           | 100%                |
| Linezolid      | -      | 100%         | -            | 100%         | -          | -           | -                   |
| Nalidixic Acid | -      | -            | -            | -            | 0%         | -           | -                   |
| Chloramphenicol| -      | -            | -            | -            | 0%         | -           | -                   |
| Clindamycin    | -      | -            | -            | 100%         | -          | -           | -                   |
| Erythromycin   | -      | -            | -            | 100%         | -          | -           | -                   |
| Cefoperazone   | -      | -            | -            | -            | -          | 100%        | -                   |
| Aztreonam      | -      | -            | -            | -            | -          | 0%          | -                   |

Discussion
The present study was carried out on 100 patients who were admitted and underwent cholecystectomy in our unit over a period of one year. The patients studied were from Shimla, its adjoining areas and referred cases from all over Himachal Pradesh.

Diseases of the gall bladder are quite prevalent in Northern India. In India, exact figures of overall incidence are not available. A bacterial cause in pathogenesis of cholelithiasis has been proposed since 1892 when Naunyn emphasized the importance of infection in the causation of gallbladder disease and advanced his theory ‘Lithogenic Cattarh’ of the gallbladder.\(^\text{(1)}\) The pathogenesis of bacterbilia is incompletely understood. The theories which have been proposed include enterohepatic route, ascending biliary tract route from duodenum and haematogenous route.

In our study, most of the cases undergoing surgery were in the age group of 31-60 years and the incidence of cholelithiasis was highest in 5\(^\text{th}\) decade. Similar findings were found in other studies. The peak incidence of cholelithiasis in a study by Chuttani et al was between 31 to 60 years.\(^\text{(3)}\) Sabharwal et al\(^\text{(4)}\) and Fazal et al\(^\text{(5)}\) found highest incidence in 5\(^\text{th}\) decade.

In the present study there were 19 males and 81 females with male to female ratio of 1:4.2. Female predominance has been observed by many workers.\(^\text{(3,4,5)}\) It has been suggested that endogenous estrogens and progesterone are responsible for the higher incidence in females, through an effect on bile saturation and smooth muscle function of gall bladder and intestine during the phases of menstrual cycle and pregnancy.

Often bile from patients with gallstone is sterile but organisms have been cultured from centre of gallstone. The radiolucent centre of many gallstones may represent mucus plugs originally formed around bacteria. Moynihan’s aphorism states that “A gallstone is a tombstone erected to the memory of organism within it”.\(^\text{(6)}\) The role of infection is unclear, whether infection causes gallstone or infection is sequelae of gallstone.

In our study out of a total of 100 patients, bile culture was found positive in 22% of patients after culture. Escherichia coli was found to be the most common organism isolated, present in 64% of samples. Enterocococcus was the second most common organism found in 13.5% of the patients.
Enterobacter, Staphylococcus, Citobacter, Salmonella and Pseudomonas were isolated in one sample each. Our results were similar to studies published by Ballal et al in 2001,\(^7\) Devangan et al in 2015,\(^8\) Gill et al in 2016\(^1\) has reported a slightly higher incidence of bacterbilia of about 30-35%. In a study by Capoor et al (2008),\(^9\) most common organism was isolated was E. coli followed by Klebsiella pneumonia, Citrobacterfreundii and Salmonella. A number of other authors (Bhansali et al in 1985,\(^10\) Cristina et al in 2003 \(^1\) and Fazal et al in 2014) have reported E Coli to be the most common organism isolated from bile. The reason for E. Coli being the commonest bacteria in bile is because it is the commonest bacteria found in GIT and infection to biliary system comes from the GIT.

E. Coli was found 100% sensitive to cefepime, cotrimoxazole, ceftriaxone, gentamicin, tobramycin and imipenem. Bhansali et al reported E coli to be sensitive to Ampicillin, Gentamicin, Cephalosporins and cotrimoxazole.\(^10\) Cristina et al reported that all gram negative organisms were sensitive to gentamicin.\(^11\)

Fazal et al reported high sensitivity of E. Coli to cefuroxime and ceftriaxone and showed high resistance to amoxicillin and ciprofloxacin.\(^5\)

**Conclusion**

Bile is generally sterile but might be infected in cholelithiasis. Incidence of bacterbilia varies from 10% to 70%. Incidence of bacterbilia is low in uncomplicated cholelithiasis whereas it is higher in complicated cholelithiasis cases due to obstruction and stasis.

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