Bile Bacteria of Patients with Cholelithiasis and Theirs Antibiogram

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Abstract- To prevent post cholecystectomy infection, the most common microorganisms causing it and their antibacterial susceptibility pattern should be determined. Therefore, the aim of the present study was to determine the exact incidence and nature of the microbial flora in the bile of the patients with cholelithiasis and chronic cholecystitis as well as their antibiotic sensitivity pattern. In this study, a total of 132 samples from the patients were tested for bacterial strains using the appropriate methods for testing them. The isolated bacteria were subsequently subjected to antibacterial susceptibility test using Kirby-Bauer method. The data were analyzed using Frequency, Chi-square and t-test. Fifty of 132 (37.87%) studied patients were positive for bacteria. The most common isolated organisms were *Escherichia coli* (13; 26%), *Enterobacteriaceae* (9; 18%), and *Salmonella typhi* (7; 14%). The most effective antibiotics were sequentially Amikacin, Ceftriaxone, and Clindamycin. Isolating bacteria and determining their sensitivity to different antibiotics may be help physicians take prophylactic measures against postoperative infection of cholelithiasis and chronic cholecystitis.

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Keywords: Antibacterial susceptibility pattern; Bacteria; Cholelithiasis

Introduction

As one of the most common diseases of gastrointestinal tract, cholelithiasis occurs in 10% of the western country's population and 17% of the Asian country's population, with an overall prevalence of 11-36%. In Iran, there are no reliable data about the overall prevalence of cholelithiasis and chronic cholecystitis. Symptomatic cholelithiasis may lead to serious complications or mortality. The most prevalent complication of cholelithiasis is chronic cholecystitis that usually requires cholecystectomy (1, 2). The biliary tract is usually sterile; however, if cholelithiasis occurs, different microbes might be identified in and/or cultured from the bile or gallbladder wall (3,4). Microscopic examinations indicated that 20-50% of the patients with chronic cholecystitis have a positive bile culture (5,6). Different reasons for biliary tract infection have been presented, *e.g*. ascending infection due to reflux of duodenal contents, blood-borne infection and infection spread through the portal-venous channels. Ascending infection from the duodenum is thought to be the primary mechanism by which bacteria enter the bile (7). Reviewing the literature, there are conflicting reports regarding the significance of bacterial infection in patients with chronic cholecystitis and cholelithiasis (8).

As prophylaxis, a number of antibiotics could be prescribed to prevent potential post-cholecystectomy infections. Thus, understanding the most common organisms causing them and their antibacterial susceptibility pattern would be useful in prevention of these infections. The present study was carried out to achieve this aim.

Materials and Methods

In this cross sectional study, 132 patients with cholelithiasis and chronic cholecystitis without risk factors for postoperative sepsis (older than 70 years old, previous biliary tract operation, jaundice, chills and fever within 1 month of cholecystitis acute attack) were subjected to elective laparoscopic cholecystectomy from September 2009 to September 2010. Using a questionnaire, patients' age, sex, clinical features of the patients, the isolated bacteria, and antibiogram were recorded. Since inadvertent entry into the gallbladder during laparoscopic cholecystectomy is not uncommon and could lead to spillage of bile or stone into the

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peritoneal cavity, all our patients received antimicrobial prophylaxis with 1g Keflin preoperatively and patients who had diabetes mellitus or spillage of bile or stone into the peritoneal cavity continued receiving 1g Keflin every 6 hours postoperatively.

During surgery 5-10 ml of the bile samples was aspirated with a sterile syringe from gallbladder immediately after cholecystectomy; 3-5 ml was placed in a sterile container; another 3-5 ml was inoculated directly into an aerobic and anaerobic BacT/Alert blood culture bottle (Organon Teknika, Durham, NC, USA). The Samples were sent immediately to the Microbiology Laboratory of Medical Faculty and incubated at 37°C for 24 h. In the laboratory, the samples were cultured in thioglycolate medium to detect anaerobic and other bacteria and were cultured on blood agar and MacKoncky agar incubated in 37°C for 24 hours. Differential tests such as IMVIC (Indole, Methyl Red, Voges presquare, and Citrate), motility, urease, and lysine decarboxylase were performed after staining and microscopic observation.

After the bacteria were isolated, we performed antibiotic sensitivity tests by the isolates carried out using Kirby-Bauer method with a colony in Mueller-Hinton agar medium (pH: 7.2-7.4) (Merck, Germany). The agar was prepared and sterilized according to the manufacturer's instructions.

The results were reported as susceptible, resistant and intermediate based on the diameter of the clear zone around disks with reference to the antibiotic standard table.

The applied antibiotic disks were Penicillin, Ampicillin, Amikacin, Gentamicin, Cefotaxime, Cefepime, Erythromycin, Clindamycin, Vancomycin, Tetracycline, Novobiocin, Ceftriaxone, Imipenem, Co-trimoxazole and Ciprofloxacin.

Results

In this study, 132 patients hospitalized due to cholelithiasis and biliary colic were included. All of the patients had cholelithiasis and 74 patients had chronic cholecystitis. The study population included 88 (66.7%) female and 44 (33.3%) male with a mean age of 55.6±14.3 years (range; 18 and 63 yr). Among the total 132 bile samples, bacteria were isolated in 50 samples (37.87%). Mean age of patients with positive and negative cultures were 56.9±14 and 52.7±14.7 years respectively, having significant difference based on Student's t-test ($P<0.05$). Frequency of isolated bacteria in females and males had no significant difference ($P>0.05$). Monomicrobial and polymicrobial infection were observed in 47 (94%) and 3 (6%) respectively. Anaerobic bacteria were detected in 8 (16%) patients; *Bacteroides fragilis* (2; 4%) and *Bacteroides perfringens* (6; 12%). *Escherichia coli* was the most common isolate (13; 26%). Enterobacter was isolated from 9 samples (18%) followed by *Salmonella typhi* (7; 14%), Coagulase-negative *Staphylococcus* (6; 12%), *Klebsiella pneumoniae* (2; 4%), *Proteus* (2; 4%). *Salmonella typhi* was isolated only from samples of female patients (Table 1).

Antibiotic susceptibility tests were performed for organisms that were isolated from the bile samples and the following data were obtained. *E.coli* was 92.3% susceptible to Amikacin, while it was nearly resistant to Erythromycin (90%). *Enterococcus was* 69.23% susceptible to Ceftriaxone, while it was 46.12% resistant to Gentamicin, Penicillin, and Cefotaxime.

Table 1. The distribution of various microorganisms.

Microorganism	Total number	Percentage
Monomicrobial		
E.coli	13	26
Enterobacter	9	18
Salmonella typhi	7	14
Coagulase-negative *Staphylococcus*	6	12
Klebsiella pneumoniae	2	4
Proteus	2	4
Anaerobic bacteria	8	16
Total	47	94
Polymicrobial		
Proteus with *Klebsiella*	1	2
E. coli with anaerobic bacteria	2	4
Total	50	100

Table 2. Antimicrobial* susceptibility of isolates in the study.

Isolate(N)	P	Amp	Ctz	Ci	Cp	Im	Ak	G	Ery	Cli	Van	Cf	Sxt	Tet	Nov
E. coli	6	6	3	7	6	9	12	7	1	8	1	9	7	4	3
Coagulase-negative *Staphylococcus*	1	1	5	1	2	2	1	7	3	1	2	2	2	2	1
Klebsiella pneumoniae	1	1	2	2	2	2	3	1	2	3	2	2	3	1	1
Salmonella typhi	2	1	6	3	4	1	3	2	4	8	7	0	5	7	8
Enterobacter	3	1	6	9	5	8	4	4	6	0	1	6	0	1	0
Proteus	0	0	2	2	2	3	3	0	1	3	2	2	0	3	3
Total	13	10	24	24	21	25	26	21	17	23	15	21	17	18	16

*P=Penicillin; Amp=Ampicilin; Ctz=Cefotaxime; Ci=Ceftriaxone; Cp=Cefepime; Im=Imipenem; Ak=Amikacin; G=Gentamycin; Ery=Erythromycin; Cli=Clindamicin; Van=Vancomycin; Cf=Ciprofloxacin; Sxt=Co-trimoxazole; Tet=Tetracyclin; Nov=Novobiocine.

The most susceptible antibiotic for microorganisms on the whole was Amikacin (52%). The antibiogram of the isolates are also summarized (Table 2). We did not have any infective complications.

Discussion

In our study the mean age of patients was 55.6±14.3 years (range; 18-63 yr), nearly in line with 46 and 51.7 years in two other studies (1, 9). From the total 132 bile samples, bacteria were isolated in 50 samples (37.87%). Some other studies have shown that 20-50% of the patients with chronic cholecystitis have a positive bile culture (5, 6). Mean age of patients with positive and negative cultures was 56.9±14 and 52.7±14.7 years respectively, having significant difference based on *t* student test ($P<0.05$). A study by Al Harbi *et al*. has shown being older than 50 years was the only factor significant in view of preoperative positive bile culture (8), which is in agreement with our study. Anaerobic bacteria were detected in 8 (16%), monomicrobial infection in 47 (94%), and polymicrobial infection in 3 (6%) patients. In a study by Ballal *et al*. in India, bile cultures for aerobic and anaerobic bacteria were carried out on 125 samples from patients with chronic cholecystitis with cholelithiasis; 71 (56.8%) aerobic and 17 (13.6%) anaerobic bacteria were detected. Among the mixed flora, 2 had only aerobes and the remaining had both aerobes and anaerobes (10). Al Harbi et al.'s study also showed polymicrobial infection in 4 (3.57%) and anaerobic bacteria in none of the cases (8). Anaerobic bacteria may grow when the bile duct is seriously infected by anaerobic and aerobic bacteria and hence body's immunity becomes low. Lou *et al*. suggested the paucity of anaerobes in the human biliary system (11). The effect of anaerobic bacteria on bile pigment stone has been reported widely (12, 13), according to which the anaerobes such as *B. fragilis* and *B. fusiformis* may produce an *E.coli* substance called β-Lactamase which resolves bilirubin. The bilirubin when integrated with calcium ion forms calcium bilirubinate.

The difference in anaerobic and polymicrobial positive culture between our study and others' could be attributed to the method of antibiotic therapy; here, we ordered antibiotic prophylaxis routinely while in Ballal *et al.* study and Al Harbi *et al*. study were selectively it , we had not known.

In our study *E. coli* was the most common isolate (13; 26%), as previously reported (14-17). Enterobacter was the second one (9; 18%) followed by *Salmonella typhi* (7; 14%), Coagulase-negative *Staphylococcus* (6; 12%), *Klebsiella pneumoniae* (2; 4%) and *Proteus* (2; 4%). The significance of *E. coli* dominance is also supported by previous reports indicating a potential role for *E.coli's* glucoronidase enzymatic activity in formation of calcium bilirubinate gall stone (18, 19). In our study, *Salmonella typhi* grew in 18% cases, sensitive to Clindamicin and Novobiocine in 88.8%. The prevalence of *Salmonella typhi* in bile of cholelithiasis patients varied widely from 1% to 34% (20, 22), perhaps due to typhoid fever which is, similar to some parts of our country, endemic in some regions. Analysis of our patients showed that *Salmonella typhi* was more common in females compared to males. Their high incidences in females have been attributed to hormonal effects related to menstrual cycle and pregnancy (10). Eslami *et al.* study conducted in Iran indicated *E. coli* (25%) as the most prevalent isolated bacterium, and the prevalence of *Klebsiella, Aerobacter, Pseudomonas, Enterococci*, and *Proteus* as 12%, 10%, 9%, 8%, and 3% respectively (23). In Ballal *et al*. study, *E. coli* and *Klebsiella* were predominant among the aerobes with 45.07% and 25.35% prevalence respectively and *B. fragilis* (58.82%) predominant among the anaerobes (10). According to Al Harbi *et al*., the most common organisms isolated were *E. coli* (26.1%), *Enterococcus*

facials (15.6%), and *Pseudomonas aeruginosa* (9.6%) (8). We do not know why our findings were not completely similar to others' studies. However, we support taking cultures of the bile at cholecystectomy because appropriate antibiotics can be administered in case of cultures being positive, hence avoiding serious complications, *e.g.* gram negative sepsis. In our study *E. coli* was 92.3% susceptible to Amikacin and Enterococci 69.23% susceptible to Ceftriaxone. The most susceptible antibiotic for microorganisms on the whole was Amikacin. Ballal *et al.* have shown anaerobes were sensitive to Cefotaxime, Metronidazole, Chloramphenicol, Cefazolin, and Tetracycline, and aerobes isolated to Ampicillin, Chloramphenicol, Streptomycin, Tetracycline, Gentamicin, and second generation Fluoroquinolones such as Ciprofloxacin and Norfloxacin (10).

In spite of the fact that we ordered antibiotic prophylaxis for all the patients, bacterial isolates and infectious complications showed no significant difference. Thus, we recommend starting antibiotics selectively, if supported by the clinical conditions and/or culture reports, in case of cholecystitis and cholelithiasis. However, routine culture of all bile samples is strongly advised.

Given our and others' studies anaerobes are rare in the human biliary system; therefore, if antibiotic therapy is considered, aerobic coverage should be satisfactory. In addition because the most susceptible antibiotic for microorganisms on the whole was Amikacin, this antibiotic can be started selectively.

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