

Full Length Research Paper

Correlation between biliary bacteriology and types of gallstones in uncomplicated symptomatic cholelithiasis

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The aim of this study was to identify the microflora in the gallbladder of patients undergoing cholecystectomy for different types of gallstones and the antibiotic susceptibility pattern of the isolates. Between January 2005 and December 2008, 136 samples of bile [cholesterol stones (CS)] and 175 pigment stones (PS) were collected from 213 women and 98 men, who were between 18-95 years (median age: 52.5±12.0) by laparoscopic or open cholecystectomy at the University Surgical Unit Duzce in Turkey. The bile samples were aerobically cultured to assess microflora and their antibiotic susceptibility. 210 (54%) of the 311 patients with gallstones had bacterial isolates; 78 isolates (37.2%) were from cholesterol stone-containing bile and 132 isolates (62.8%) from pigment stone-containing bile ($P<0.01$). The overall bacterial isolates from bile samples revealed *Escherichia coli* (*E. coli*) predominantly, followed by *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Enterococcus* spp. In one sample, we found *Candida lusitanae* (*C. lusitanae*). None of the Gram negative strains were resistant to antibiotics but Gram positive strains were highly resistant to penicillins (100%). Looking at the microflora of the gallbladder and the susceptibility pattern of our isolates, we would suggest that antibiotic prophylaxis recommended for laparoscopic or open cholecystectomy for gallstones be reviewed and the role of bacteribilia in the surgical management of cholelithiasis requires further study.

Key words: Microflora, bile culture, gallstones, cholecystectomy.

INTRODUCTION

Gallstone disease is a common and costly disorder worldwide; it is often asymptomatic, biliary colic, cholecystitis and entails life-threatening complications such as obstructive jaundice (Stringer et al., 2013). The pathophysiological role of bacteria in the formation of gallstones was proposed long ago. Bacteria are often

found in high concentrations in pigment and less in cholesterol gallstones. Although it is intriguing to hypothesize that cholesterol stones (CS) formation is non-bacterial in nature and essentially different from the pathogenesis of "infectious" pigment stones (PS), it is more likely that significant overlap exists between the two

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processes. This is in keeping with the fact that most gallstones are mixed in nature (Swidsinski and Lee, 2001).

Cholecystectomy is among the top 10 surgical procedures performed each year in Western societies; 2 with more than 500 000 patients are performed annually in the United States, consuming resources estimated at \$6.5 billion (Stringer et al., 2013). In the present study, we demonstrated that gallbladders excised from patients with symptomatic cholelithiasis, even without any evidence of inflammation, might host bacteria in over 20% of cases (Gosling et al., 1996). Prompt administration of the appropriate antibiotics is crucial in the management of biliary tract infection and antimicrobial treatment is commonly administered pre- or peri-operatively and often inhibits bacterial growth. Moreover, it is suggested that the recovery of bacteria in bile cultures is affected by toxicity of bile salts. Thus, traditional culturing methods of bile might miss a large number of underlying bacterial infections that could lead to acute or chronic cholecystitis (Grill et al., 2000; Cheng et al., 2002; De Boever and Verstraete, 1999; Kurtin et al., 2000; Manolis et al., 2008). Culture of bile from the gallbladder of patients with uncomplicated cholelithiasis during cholecystectomy has shown principally *E. coli*. Others include *Pseudomonas* spp., *Enterococcus faecalis*, *Streptococcus* spp. and *Klebsiella* spp. (Darko and Archampong, 1994; Gold-Deutch et al., 1996).

Bile from the gallbladder of patients with uncomplicated cholelithiasis who have different types of gallstones is rarely studied. Also, investigations of type and prophylactic antibiotic sensitivity of microflora in bile from the gallbladder are rare in patients with different types of gallstones. This study aimed to determine the nature of microorganisms and the antimicrobial susceptibility of bacteria in bile from patients with uncomplicated symptomatic cholelithiasis who have different types of gallstones.

MATERIALS AND METHODS

Patients

Between January 2007 and December 2010, samples of bile (136 cholesterol stones and 175 pigment stones) were obtained from 311 patients (213 women and 98 men; aged 18-95, with a median age of 52.5±12.0 years) by laparoscopic (85.9%) or open cholecystectomy (3.8%) at the University Surgical Unit Duzce, Turkey. Cholelithiasis was diagnosed by abdominal ultrasonography or computer-aided topographic scan. The patients were followed up for a week and a month after being discharged from the surgical clinic.

For the selection of patients, excluded from the study were those who had previous episodes of cholecystitis or cholangitis.

Selection criteria for gallstones

Gallstones were classified according to visual appearance or color. The stones were separated into two groups: (1) black stones and brown stones were regarded as PS, (2) yellow stones were regarded as CS.

Collection of bile

As the gallbladder was removed at surgery, it was punctured under sterile conditions and 2.0 ml of bile was aspirated. The sample was transferred to a sterile container and transferred at room temperature in an hour to the laboratory for culture.

Method of culture and identification of bacterial colonies

Bile samples were cultured aerobically in blood agar (Oxoid, Basingstoke, UK), EMB agar (Difco, Detroit, Mich., USA) and Sabouraud dextrose agar (Difco, Detroit, Mich., USA). Direct inoculates of culture media and plates were incubated at 37°C under aerobic conditions. Direct gram staining was done to test for the presence of organisms and pus cells. The culture plates were examined at 24 and 48 h for bacterial growth. An aliquot of bile was inoculated into brain heart infusion (BHI) as an enrichment procedure. The broths were sub-cultured onto solid media following 24 h of incubation at 37°C. The plates were examined for bacterial growth at 24 and 48 h. Bacterial growth was identified by gram staining, culture characteristics, and biochemical test (API 32 E and API 32 GN, and kits, Bio- Merieux SA, Marcy l'Etoile France).

Antibiotic sensitivity test

Antibiotic sensitivity testing of isolates was performed using Clinical Laboratory Standard Institution (CLSI) methods (CLSI 2012). For Gram-negative isolates, the following antimicrobials were used: ampicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), amikacin (30 µg), gentamicin (10 µg), ceftazidime (30 µg), ceftioxone (30 µg), cefotaxime (30 µg), cefepime (30 µg), ceftazidime (30 µg) and imipenem (10 µg); for Gram-positive isolates: oxacillin, penicillin (10 µg), vancomycin (30 µg), erythromycin (15 µg), ciprofloxacin (5 µg), co-trimoxazole (1.25/23.75 µg), gentamicin (10 µg), amikacin (30 µg), Clindamycin (2 µg), chloramphenicol (30 µg) and Rifampicin (30 µg).

Statistical analysis

Data were expressed as frequencies and analyzed using SPSS (SPSS 18.0, Chicago, Illinois, USA). The statistical significance of the incidence of positive and negative cultures from the patients and controls were compared using the paired *t* test. Significance was assigned a P value of <0.05.

RESULTS

In the 311 bile samples, 210 (54%) were shown to have bacterial isolates. 78 (37.2%) isolates were from CS-containing bile and 132 (62.8%) were from PS-containing bile ($P<0.05$). Over 50 years and sex were the significant pre-operative factors associated with positive bile culture containing stones ($P<0.05$). The overall bacterial isolates from bile samples showed 54.7% (115/210) *E. coli*, 23.3% (49/210) *P. aeruginosa*, 12.8% (27/210) *Enterococcus* spp., 3.9% (8/210) *Klebsiella* spp., 2.8% (6/210) *Staphylococcus epidermidis* (coagulase-negative) and the other microorganisms (*S. aureus*, *Streptococcus* spp., *Candida* spp.) (Table 1). In one sample, we found *C. lusitaniae*. No bile samples from the controls showed bacterial growth after direct inoculation or after BHI

Table 1. Incidence of Bacteria in CS and PS- containing bile.

Microorganisms	Cholesterol stone containing bile (n/%)	Pigment stone-containing bile (n/%)	All (n/%)
<i>E.coli</i>	51/44.4	64/55.6	115/54.7
<i>P.auregenosa</i>	22/44.9	27/55.1	49/23.3
<i>Enterococcus spp.</i>	10/37.1	17/62.9	27/12.8
<i>Klebsiella.spp</i>	3/37.5	5/62.5	8/3.9
<i>S.epidermidis</i>	3/50.0	3/50.0	6/2.8
Other m.o*	2/44.4	4/66.6	6/2.4

**S. aureus*, *Streptococcus spp.*, *Candida spp.*

Table 2. Antibiotic sensitivity pattern of Gram negative organisms (%).

Antibiotics	Cholesterol stone- containing bile			Pigment stone-containing bile		
	S	MS	R	S	MS	R
Amikacin	100			100		
Ampicillin	98	2		98	2	
Ceftriaxone	98	2		100		
Cefotaxime	100			100		
Ceftriaxone	100			100		
Cefepime	100			100		
Ceftazidime	99	1		98	2	
Choramphenicol	98	2		99	1	
Ciprofloxacin	100			100		
Gentamicin	100			100		
Imipenem	100			100		

S=Sensitive; MS= Moderately sensitive; R=Resistant.

enrichment. Polymicrobial infection was encountered in 4.7% (10/210) and did not show any difference in the bile containing CS and PS ($P>0.00$).

Gram negative and Gram positive bacterial isolates from the bile containing CS and PS did not show any difference in sensitivity to antibiotics (Tables 2 and 3) ($P=0.01$). None of Gram negative bacterial isolates were resistant to antibiotics but Gram positive bacterial isolates were highly resistant to Penicillins (100%) (Tables 2 and 3).

DISCUSSION

There are conflicting reports in the literature regarding the significance of bacterial infection in both normal subjects and patients with cholelithiasis. It has been suggested that the discordant reports were because of the differences in grouping of patients, in sampling and cultural techniques (Csendes et al., 1996a).

Bile cultures during biliary surgery for patients with risk factors include age above 70 years, previous biliary tract operation, jaundice, chills and fever within one week of operation, and operation performed within one month of an acute attack of cholecystitis (Csendes et al., 1996a; Csendes et al., 1975; Nichols, 1984). Antimicrobial prophylaxis is recommended when one or more of these risk factors are identified preoperatively, although their relative importance in laparoscopic cholecystectomy is undetermined (Nord, 1990; den Hoed et al., 1998). In our study, over 50 years and sex were the significant pre-operative factors associated with positive bile culture containing stones ($P<0.01$). Isolation rates of bacteria in the bile of patients with gallstones ranging from 3 to 72% have been reported in the literature (Csendes et al., 1996a; Csendes et al., 1975; Won et al., 2008; Mahafzah et al., 2009).

The role of bacteria in the pathogenesis of CS and PS-containing bile has been rarely studied. Direct evidence of bacterial presence is not sufficient because of failure to

Table 3. Antibiotic sensitivity pattern of Gram positive organisms (%).

Antibiotics	Cholesterol stone- containing bile			Pigment stone-containing bile		
	S	MS	R	S	MS	R
Amikacin	100			100		
Chloramphenicol	100			100	2	
Ciprofloxacin	99	1		100		
Clindamycin	100			100		
Methicilline/Cloxacillin	100			100		
Erythromycin	100			100		
Gentamicin	100			100	2	
Penicillin	100			100	1	
Rifampicin			100		100	
Co-trimoxazole	100			100		
Vancomycin	100			100		

S=Sensitive; MS= Moderately sensitive; R=Resistant.

culture bacteria from CS (Darko and Archampong, 1994). Bacteria are often found in high concentrations in pigment and less in cholesterol gallstones. Although it is intriguing to hypothesize that CS formation is non-bacterial in nature and essentially different from the pathogenesis of "infectious" PS, it is more likely that significant overlap exists between the two processes. This is keeping with the fact that most gallstones are mixed in nature (Swidsinski, 2001). According to our study, In 311 bile samples, 210 (54%) were shown to have bacterial isolates, 78 (37.2%) isolates were from CS-containing bile and 132 (62.8%) were from PS-containing bile ($P=0.01$).

The majority of the organisms isolated were enteric coliforms and *P. aeruginosa* followed by Gram positive bacteria such as Enterococcus, Staphylococcus, Streptococcus (Bistgani and Imani, 2013; Li and Ji, 2009; Sattar et al., 2007; Flores et al., 2003). Similar to our study, the most common organisms were *E. coli*, *P. aeruginosa*, *Enterococcus* spp., *Klebsiella* spp. and *Staphylococcus epidermidis* (coagulase-negative). Enteric organisms have often been suspected to cause cholelithiasis, and intestinal flora has frequently been recovered followed by intervention on the biliary tree (Abd-Alkareem, 2011).

An interesting finding in this study is the identification of *C. lusitaniae*. Candidial cholecystitis is seen especially in patients with malignancies. According to Yildirim et al. (2008), in the review of the literature, this is the first report on acut calculous cholecystitis by *C. lusitaniae*. *C. lusitaniae* is intrinsic or secondary resistance to amphotericin B. This patient was treated with fluconazole.

No scientific data support antibiotic treatment of biliary microflora. There are conflicting reports on the usefulness of bile cultures at the time of surgery and the association

of positive bile cultures with post-operative wound infection and septic complications (Darko and Archampong, 1994; Gold-Deutch et al., 1996; Abeyesuriya et al., 2008). There is a higher incidence of postoperative morbidity and infectious complications in patients with pathogenic bacteria in gallbladder bile than in patients with no bacterial growth or opportunistic bacteria (Csendes et al., 1996).

Antibiotic sensitivity patterns of bacterial isolates in the bile of patients in this study were similar to those reported elsewhere. Gram negative and Gram positive isolates from the bile containing CS and PS did not show any difference in sensitivity to antibiotics ($P=0.01$).

Bistgani and Imani (2013) in their studies concluded that the most susceptible antibiotic for microorganisms on the whole was Amikacin but our studies show that none of Gram negative bacteria were resistant to antibiotics and Gram positive bacteria were highly resistant to penicillins (100%).

Vancomycin and teicoplanin showed 100% sensitivity. Antibiotic sensitivity patterns of isolated organisms were similar, irrespective of the type of stone in the bile. Imipenem and vancomycins may be used as second choice to treat the infection which other drugs cannot treat.

In conclusion, bacterial isolates were significantly more common in PS-containing bile than in CS-containing bile in this study.

Our study shows an association between gallstone formation and the presence of bacteria in bile. From the microflora of the gallbladder and the susceptibility pattern of our isolates, we would suggest that antibiotic prophylaxis recommended for laparoscopic or open cholecystectomy for gallstones needs to be reviewed and the role of bacteribilia in the surgical management of cholelithiasis requires further study.

Conflict of interests

The authors have not declare any conflict of interest.

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