

Isolation and identification of bacteria from patients with cholecystitis and cholelithiasis undergoing cholecystectomy

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Abstract

Fourty patients were included in this study, which was extending from April 2008 till October 2008. The patients were attending the Al-Sader Teaching Hospital in Al-Najaf province. They were all undergoing clinically and ultrasound examination. 16 of them were with cholecystitis and 24 were with cholelithiasis. 11 were males and 29 were females. The age range was 15-65 years. All patients were undergoing cholecystectomy. Bile fluid and pieces of gallbladder were collected from each patient and cultured on routine bacteriological media. Identification of bacterial growth was based on standard bacteriological criteria. The results revealed that all patients were culture positive. *Escherichia coil* represent the most common isolates (75%) in cholecystitis, and (79.17%) in cholelithiasis, while β -hemolytic streptococci were found in (12.5%) of cholecystitis cases and (8.33%) of cholelithiasis cases. *Klebsiella* spp. were found in (6.25%) of cholecystitis and (12.5%) of cholelithiasis cases. *Proteus* spp. were isolated from (6.25%) of patients with cholecystitis. It can be concluded that the isolation rate of Gram's negative bacteria were higher than that of Gram's positive bacteria from patients with cholecystitis and cholelithiasis undergoing cholecystectomy.

Introduction:

Cholecystitis refers to a painful inflammation of the gallbladder's wall , that may occur as acute or chronic. Acute cholecystitis is one of the most common and major complication of gallstones (Chatziioannou *et al.*, 2000). Gall stones are solid accumulations of the components of bile , particularly cholesterol , bile pigments and calcium (Irfans *etal.*,2007). Gallstone disease (Cholelithiasis) remains one of the most common medical problems leading to surgical intervention. Cholelithiasis affects approximately 10% of the adult population in the United States (Schirmer *et al.*, 2005). The gallbladder is often colonized by Salmonella during typhoid fever causing acalculous cholecystitis (Ruiz-Rebollo *et al.*, 2008) . It has been demonstrated that infected gallbladders showed histopathological damage characterized by destruction of the epithelium and massive infiltration of neutrophils, accompanied by a local increase of proinflammatory cytokines (Menendez *et al.*, 2009). Furthermore, Most of the studies have shown a good association of mixed bacterial and Salmonella infections in the carcinogenesis of cancer gallbladder especially in the area of high endemicity of typhoid (Kumar, 2006). In Saudi Arabia, 25% of patients undergoing laparoscopic cholecystectomy for gallstones were bacterial culture positive, and the most common organisms isolated were *E. coli* (28.1%), *E.faecalis* (15.6%) and *P. aeruginosa* (9.4%). No anaerobes were detected, but *Candida albicans* was isolated in one case (Al Harbi *et al.*, 2001). In another study among patients undergoing cholecystectomy, The overall, bile cultures were positive in 22%. There were 13.3% patients with positive bile from the gallbladder. From the laparoscopically operated patients 2.8% had a positive bile culture. The predominant microorganisms from gallbladder bile were *E. coli*, *Klebsiella* spp. and *Streptococcus* spp. There was no

relationship between positive gallbladder cultures and wound infection (Den Hoed *et al.*, 1998).

Capoor *et al.* (2008), in a study to determine the spectrum of biliary microflora in patients with acute cholangitis with and without cholelithiasis or other biliary diseases demonstrated that bacteria were recovered from 32% of patients with cholecystitis with cholelithiasis, 51.4% in patients with acute cholecystitis, and 1.6% in patients with gallbladder carcinoma. The most common organisms isolated were *E. coli* (29.7%), *K. pneumoniae* (27%), *C. freundii* (8.1%), *S. enterica* serovar Typhi (8.1%). The frequencies of gram-negative aerobic bacteria were high among patients with primary biliary infection, while those of gram-positive aerobic bacteria were high among patients with postoperative biliary infection. Although vancomycin-resistant enterococci was not isolated, the frequency of Methicillin-resistant *S. aureus* was extremely high (Mukaiya *et al.*, 2005). In a study included 68 patients (41 with cholelithiasis and 27 with cholecystitis), *H.pylori* DNA was detected in 15% of the patients in gallbladder tissue specimens, but no clinical correlation with biliary disease could be established (Yucebilgili *et al.*, 2009). Recently, it has been found that PCR is more sensitive in detecting bacterial contamination of gallbladder bile in patients with chronic cholecystitis than conventional culture method (Lemos *et al.*, 2010).

Materials and Methods:

Forty patients were included in this study, which was extending from April 2008 till October 2008. The patients were attending the Al-Sader Teaching Hospital in Al-Najaf province.

All patients were undergoing cholecystectomy. Bile fluid and pieces of gallbladder were collected aseptically from each patient using sterile universal bottles containing Brain Heart infusion broth as transport media. Specimens were culture on blood agar and MacConkey agar plates as soon as it delivered to the laboratory, and incubated overnight at 37 °C. Identification of bacterial growth was based on colonial morphology and standard biochemical tests (Collee *et al* ,1996).

Results:

They were all undergoing clinically and ultrasound examination. 16 (40%) of them were with cholecystitis and 24 (60%) were with cholelithiasis. 11 (27.5%) were males and 29 (72.5%) were females. The age range was 15-65 years. The male patients consist of 5 (12.5%) with cholecystitis and 6 (15%) with cholelithiasis, while the female patients consist of 11(27.5%) with cholecystitis and 18(45%) with cholelithiasis, table (1).

Table (1): distribution of clinical cases according to the age and sex of patients.

Age	Male	Female	Cholecystitis		Cholelithiasis	
			Male	Female	Male	Female
15- 25	2	2	1	2	1	0

26-35	7	12	3	3	4	9
36-45	1	9	1	2	0	7
46 +	1	6	0	4	1	2
Total	11	29	5	11	6	18
Percentage	27.5%	72.5%	12.5%	27.5%	15%	45%

All patients were culture positive. The type and percentage of bacterial isolates in cases of cholecystitis were listed in table (2). 16 bacterial isolates were recovered from patients with cholecystitis. The most common bacterial isolates was *E. coli* (75%), followed by β -hemolytic streptococci (12.5%), and *Klebsiella* spp. and *Proteus* spp. (6.2%) for each.

Table (2):type and percentage of bacteria isolates from patients with cholecystitis.

Bacterial isolates	Cholecystitis			
	Males	Females	Total	Percentage
<i>E.coli</i>	4	8	12	75%
<i>Klebsiella</i> spp.	0	1	1	6.25%
<i>Proteus</i> spp.	0	1	1	6.25%
β -hemolytic streptococci	1	1	2	12.5%

Regarding the cases of cholelithiasis, 24 bacterial isolates were recovered. The most common bacterial type was *E. coli* (79.17%), followed by *Klebsiella* spp.(12.5%) and β -hemolytic streptococci (8.33%), table (3).

Table (3):Type and percentage of bacteria isolates from patients with cholelithiasis.

Type of bacteia	Cholelithiasis		Total	Percentage
	Male	Female		
<i>E.coli</i>	5	14	19	79.17%
<i>Klebsiella</i> spp.	1	2	3	12.5%
<i>Proteus</i> spp.	0	0	0	0
β -hemolytic streptococci	0	2	2	8.33%

Discussion:

Most of the previous studies on cholecystitis or cholelithiasis have documented that the bacteria can be cultured from the bile or gallbladder wall, and that the biliary infections can be caused by wide spectrum of bacteria ranging from aerobic Gram's positive to Gram's negative to anaerobic organisms. Aerobic organisms cause 94% of biliary tract infection while anaerobic organisms caused the rest (Den Hoed *et al.*, 1998; Mukaiya *et al.*, 2005).

The overall bacterial isolation rate obtained from patients with cholecystitis or cholelithiasis in the present study was higher than that reported by other workers (Den Hoed *et al.*, 1998; Al Harbi *et al.*, 2001; Capoor *et al.* 2008). This is probably due to the fact that the patients in our community, in general, are usually delaying in seeking medical advice, and that may provide additional time for the development of infection. Furthermore, recurrent postponing of cholecystectomy for technical or financial reasons may also offer an opportunity for infection and other complications (Kumar *et al.*, 2006).

The present study revealed that the Gram's negative bacteria in general and *E. coli* in particular, constitute the highest isolation rate. These results are consistent with that reported by other studies (Ballal *et al.*., 2001 ; Irfan *et al.* , 2007). The high percent of *E.coli* may be due to the activity of glucuronidase enzyme which play an important role in formation of calcium bilirubinate gallstone formation (Al-Khafeji,2006). The other bacteria isolates recovered in this study are in agreement with previous studies (Ballal , 2001; Al-Harbi *et al.*,2001).

The higher susceptibility rate of females to cholecystitis and cholelithiasis compared to males are concordant with the results of another study (Irfan *et al.* .,2007), who recorded that the incidence of cholecystitis increase in females because of hormonal difference due to the effect of endogenous as well as exogenous estrogen or progesterone or both decrease the chenodeoxy cholic acid and total bile acid pools, with the consequent increase in bile cholesterol secretion and saturation.

It can be concluded that high rate of bacterial infection, particularly Gram's negative bacteria was recorded among patients with cholecystitis or cholelithiasis undergoing cholecystectomy.

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عزل وتشخيص البكتيريا من المرارة المستأصلة من المرضى المصابين بالتهاب المرارة (مصحوب بالحصى او بدونه)

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الخلاصة :-

تناولت هذه الدراسة (40) حالة مرضية جميعها كانت تعاني من حالات خمج المرارة وخرمج المرارة المصحوب بحصى المرارة ، أمتدت الدراسة من شهر نيسان 2008 الى شهر كانون الأول 2008 في مستشفى الصدر التعليمي في محافظة النجف الأشرف .كل المرضى كان يتم فحصهم سريريا وبالأشعة فوق الصوتية (السونار). 16 من المرضى كانوا يعانون من التهاب كيس المرارة 24 منهم كانوا يعانون من التهاب كيس المرارة المصحوب بحصى المرارة ، عدد الرجال كان 11 و29 نساء تراوحت أعمارهم بين 15 – 65 سنة . السائل الصفراوي وقطع من المرارة كانت تجمع من صالات العمليات بعد أستأصل كيس المرارة ثم يتم أخذ سائل الصفراء وجزء من كيس الصفراء ثم تزرع على الأوساط الزرع البكتيري الروتينية و تشخص المزراع البكتيرية بالأتماد على المعايير التشخيص القياسية .

اظهرت نتائج البحث ان نسبة البكتيريا *Escherichia.coli* في حالات خمج المرارة كان (75%) وفي حالات خمج المرارة المصحوب بحصوة المرارة كان (79.17%) بينما كانت نسبة بكتيريا *Streptococci* في حالات خمج المرارة (12.5%) وفي حالات خمج المرارة المصحوب بحصوة المرارة كان (8.33%) . وكان نسبة بكتيريا *Klebsiella spp.* (6.25%) في حالات خمج المرارة و (12.5%) في حالات خمج المرارة المصحوب بحصوة وبكتيريا *Proteus spp.* كانت نسبتها في حالات خمج المرارة (6.25) . يمكن الاستنتاج بأن نسبة عزل بكتيريا سالبة الصبغة كرام أعلى من البكتيريا ذات الصبغة كرام الموجبة في المرضى المصابين بالتهاب كيس المرارة المصحوب بالحصى أو بدون حصى.