ORIGINAL ARTICLE

Host biliary bacteria in cholecystectomy at a secondary-care teaching hospital, an observational study

Bacteriología biliar del huésped en colecistectomía en un hospital de enseñanza de segundo nivel, un estudio observacional

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Abstract

Objective: *The prevalence of cholelithiasis in Mexico is high, and cholecystectomy is a very frequent procedure where antimicrobials are employed, being important to sample and culture the bile fluid to identify local susceptibility to antibiotics to avoid complications and reduce antimicrobial resistance.* **Method:** *An observational, cross-sectional, prospective study was designed to identify bile fluid flora in patients undergoing cholecystectomy in a secondary-care teaching hospital with identification, sensitivity, and antimicrobial resistance testing.* **Results:** *There were 60 patients (47 female and 13 male), which yielded 15 cases with bactibilia. Bacterial identification was Enterococcus faecalis in 6 cases (40%), Escherichia coli in 5 cases (33.3%), Klebsiella pneumoniae in 2 cases (13.3%), and Raoutella planticola and Streptococcus infantarius, each in one patient. E. faecalis, E. coli, R. planticola, and S. infantarius were mostly sensitive to quinolones, whereas K. Pneumoniae was sensitive to carbapenems and tigecycline. Resistance occurred for ampicillin, benzylpenicillin, and trimethoprim/sulfamethoxazole.* **Conclusion:** *Based on our local microbial findings, it is proposed that in cases of acute biliary pathology in patients above the seventh decade of life, empirical antibiotic prophylaxis be used and a bile fluid sample be taken before starting gallbladder dissection, for later adjustment of antibiotic therapy.*

Keywords: *Bile, cholelithiasis. Cholecystectomy. Microbiology. Bacteriology.*

Resumen

Objetivo: *La prevalencia de colelitiasis en México es alta y la colecistectomía es un procedimiento muy frecuente, en el que es usual el manejo antimicrobiano, siendo importante cultivar el líquido biliar para identificar la sensibilidad antibiótica local, evitar complicaciones y reducir la resistencia antimicrobiana.* **Método:** *Se diseñó un estudio observacional, transversal y prospectivo para identificar la flora biliar de pacientes colecistectomizados en un hospital universitario de segundo nivel con pruebas de identificación, sensibilidad y resistencia antimicrobiana.* **Resultados:** *Se incluyeron 60 pacientes (47 mujeres y 13 hombres) y hubo 15 casos de bactibilia. La identificación bacteriana fue Enterococcus faecalis en 6 casos (40%), Escherichia coli en 5 casos (33.3%), Klebsiella pneumoniae en 2 casos (13.3%) y Raoultella planticola y Streptococcus infantarius, cada uno en un paciente. E. faecalis, E. coli, R. planticola y S. infantarius fueron en su mayoría sensibles a las quinolonas, mientras que K. pneumoniae lo fue a los carbapenémicos y a la tigeciclina. Se halló resistencia a ampicilina, bencilpenicilina y trimetoprima-sulfametoxazol.* **Conclusiones:** *De acuerdo con los hallazgos microbiológicos locales, se propone, en patología biliar aguda en pacientes mayores de la séptima década de la vida, antibioticoterapia empírica con toma de muestra de bilis antes de la disección vesicular, para posterior ajuste antimicrobiano.*

Palabras clave: *Colelitiasis. Colecistectomía. Microbiología. Bacteriología.*

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Introduction

Cholelithiasis is a multifactorial pathology determined by the complex interaction of genes and environment, characterized by an imbalance in the homeostasis of cholesterol and bile salts that causes its precipitation in the bile. It is very common in Western societies, especially in those most affected by a high incidence of obesity, and is more prevalent in women, especially after the fourth decade of life[1].

A crude prevalence of 14.3% (8.5% in men and 20.4% in women) was found in a necropsy study in Mexico City, whereas in Mexican-American subjects from a diabetic cohort in Texas, the prevalence was 7.2% in men and 23.2% in women[2,3].

Around 65-80% of gallbladder pathology is asymptomatic, whereas in up to 20-35% of cases, biliary colic is the most common clinical manifestation. Among its complications, there are acute cholecystitis, hydrocholecyst (gallbladder hydrops), gallbladder empyema, ascending cholangitis and biliary pancreatitis, where the primary goal of antimicrobial therapy is to limit both the systemic septic response and local inflammation, to prevent post-operative infection[4,5].

Surgical management for gallbladder pathology arose at the end of the 19th century, after the appearance of antisepsis (1846) and anesthesia (1867). Nevertheless, due to the technical and scientific advances, in 1985, the era of laparoscopic (LC) surgery began, considering the ambulatory LC management as the new gold standard since the last decade[6,7].

In the past century, it was widely accepted that bile fluid was sterile in conditions without biliary tract pathology[8]. However, an increasing number of studies about human microbiota have dismissed this classical postulate, since the advent of 16S ribosomal RNA sequencing confirmed the presence of microbes in bile samples otherwise considered sterile with culture-based techniques, introducing the concept of "biliary microbiota." Quantitative or qualitative changes in the composition of the microbial community (Bactibilia) can derange this equilibrium, favoring the development of diseases[9]. Furthermore, the presence of living bacteria in gallstones has been proven using electron microscopy, bacteriological cultures, and molecular genetic methods[8].

In cases of lithiasic cholecystitis, the most frequently isolated microorganisms are *Escherichia coli, Streptococcus faecalis, Klebsiella spp, Enterobacter spp*, and *Proteus*, as well as some anaerobes such as *Bacteroides fragilis* and *Clostridium perfringens*[10].

Considering cholecystectomy as a clean-contaminated procedure, empirical antibiotic therapy is often recommended in acute cases, as in the Tokyo guidelines 2018 (TG18) until the bile culture result is available to identify causative microorganisms (bactibilia) and the susceptibility testing is also available to adjust to specific antimicrobial agents[5], particularly when there are signs of acute inflammation (cholecystitis or cholangitis), becoming important to sample the bile fluid of cholecystectomized patients (urgent or elective) and reduce the possibility of antimicrobial resistance.

Method

To identify the most frequent type of bacteria in the bile fluid of patients undergoing open (OC) or LC cholecystectomy in our institution, an observational, descriptive, cross-sectional, prospective study was designed in the surgery department of a secondary-care teaching hospital. The study was conducted from October 2016 to March 2017 and included consecutive non-randomized patients of both genders, > 15 years of age who required a cholecystectomy and who agreed to participate in the study.

Cholecystectomy procedures were done as usual, accordingly to the staff surgeon's preferences. Antibiotic prophylaxis includes ceftriaxone or cefazoline upon availability, administered at the moment of induction. Cases in which the protocol for sample collection and/or transport to the microbiology laboratory was compromised were excluded from the study, as were cases presenting with large stones completely occupying the gallbladder lumen, thereby precluding bile fluid aspiration.

Intraoperative biliary samples (3 c.c. of bile fluid) were collected under aseptic conditions by direct puncture with a sterile syringe attached to a 21 gauge needle in OC or to a LC needle in LC, emptying its contents into a sterile test tube with airtight cap, for immediate shipment to the microbiology laboratory to be sown in a general culture medium (blood agar) and a selective medium (MacConkey agar) for microbiological grow and posterior identification.

The samples were grown at a constant temperature of 35°C, with a first review at 24 h and later at 48 h, to determine bacterial growth. If the sample did not show bacterial growth after 48 h, the process was repeated. In the case of samples with bacterial growth,

identification, sensitivity, and antimicrobial resistance testing were carried out using a Vitek 2 system (bioMérieux, Lyon, France) according to the manufacturer's protocol[11].

The procedures described were carried out in strict compliance with the ethical standards of the Research Ethics Committee of the hospital (CEI-0178-2016) in accordance with the Helsinki Declaration of 1975 and its last update of 2013 in Fortaleza, Brazil[12]. Likewise, written informed consent was obtained from each included patient, respecting their anonymity.

Statistical analysis

For the statistical analysis, the information was captured in a LibreOffice Calc sheet and then performed with PSPP 1.6.1 (GNU project/Free software foundation) for a descriptive analysis.

Results

Sixty patients were included over a 6-month period. Their age ranged from 19 to 84 years (mean 47 years). There were 47 women (78.3%) and 13 men (21.7%), with a 3.6:1 ratio.

No bacterial growth occurred in 45 of the cases (75%), resulting in positive cultures in 15 cases (25%). Among those patients with positive cultures, there were 7 men (46.6%) and 8 women (53.3%), with a mean age of 62 years (range 39-84 years).

The most frequent pathology was cholelithiasis in 23 cases (38.3%), followed by acute chronic cholecystitis in 19 patients (31.7%) and gallbladder empyema in 9 cases (15%) (Table 1).

Gallbladder empyema was related to a positive culture in 66.66%, followed by acute pancreatitis (50%), choledocholithiasis (25%), and acute chronic cholecystitis (21.05%). The only patient with ascending cholangitis was culture-positive (Table 1).

In all patients who underwent urgent surgery (within 12 h of admission) bactibilia was present, whereas patients with early intervention (within 12-48 h after admission) were positive in 36.84%, and those undergoing elective surgery (more than 1 week after admission) were positive in 13.15% (Table 2).

In regard to the germs grown, five types of bacteria were isolated in the 15 positive cultures (mono-bacterial growth), the most frequent isolated bacteria were *Enterococcus faecalis* in 6 cases (40%), of which three were in relation to gallbladder empyema. *E. coli* was identified in 5 cases (33.3%), all being diabetics. *Klebsiella pneumoniae* was isolated in 2 cases (13.3%), one with ascending cholangitis and the other with gallbladder empyema. *Raoutella planticola* and *Streptococcus infantarius* were each presented in a patient, both with chronic cholecystitis (Table 3).

To the bacterial sensitivity to antimicrobials, *E. faecalis, E. coli, R. planticola*, and *S. infantarius* were mostly sensitive to quinolones, whereas *K. Pneumoniae* was to carbapenems and tigecycline (Table 4), whereas resistance was more important for ampicillin, benzylpenicillin, and trimethoprim/sulfamethoxazole, although it was also reported for clindamycin, tetracycline, ciprofloxacin, and nitrofurantoin (Table 4).

No infectious complications (superficial wound, fascia, and organ space) occurred in any of the cases included in the study after a 30-day follow-up period.

Table 1. Bacterial identification from cultures

Diagnosis	Frequency (%)	Positive culture (%)	Negative culture (%)
Cholelithiasis	23 (38.3)	1 (4.35)	22 (95.65)
Acute chronic cholecystitis	19 (31.7)	4 (21.05)	15 (78.95)
Gallbladder empyema	9 (15)	6 (66.66)	3 (33.34)
Choledocolithiasis	4 (6.7)	1 (25)	3 (75)
Acute pancreatitis	4 (6.7)	2 (50)	2 (50)
Cholangitis	1 (1.7)	1 (100)	0 (0)

Table 2. Type of intervention and germ grown

Type of intervention	Frequency (%)	Positive culture (%)	Negative culture (%)
Urgent (within 12 h)	3 (5)	3 (100)	0 (0)
Early (within 12-48 h)	19 (31.7)	7 (36.84)	12 (63.16)
Elective (after 1 week)	38 (63.3)	5 (13.15)	33 (86.85)

Discussion

In this study, 25% of patients undergoing cholecystectomy because of an acute biliary pathology were identified with bactibilia, where most frequently identified bacteria were *E. faecalis, E. Coli,* and *K. pneumoniae*, as has been reported in several other studies[5,13-18]. It is generally accepted that the frequency of bactibilia in low-risk patients is < 10%,

Table 3. Relation of bacterial growth to diagnosis

Bacteria	Frequency (%)	Cholecystitits	Gallbladder empyema	Cholangitis	Choledoco-lithiasis	Pancreatitis
Enterococcus faecalis	6 (40)	1	3	0	1	1
Escherichia coli	5 (33.3)	3	2	0	0	0
Klebsiella pneumoniae	2 (13.3)	0	1	1	0	0
Raoutella planticola	1 (6.7)	1	0	0	0	0
Streptococcus infantarius	1 (6.7)	1	0	0	0	0

Table 4. Antibiotic sensitivity and resistance of positive bile cultures

Antibiotic	Isolated pathogen				
	***Enterococcus faecalis* (%)**	***Escherichia coli* (%)**	***Klebsiella pneumonia* (%)**	***Raoutella planticola* (%)**	***Streptococcus infantarius* (%)**
Antibiotic sensibility (CMI)					
Ciproflaxin	< 0.5-1 (100)	< 0.25 (60)		< 0.25 (100)	< 0.5 (100)
Moxifloxacin	≤ 0.25 (83.3)	≤ 0.25 (100)			< 0.5 (100)
Levofloxacin	1-2 (100)				< 0.5 (100)
Gentamicin		< 1 (100)			
Amikacin		< 2 (100)			
Ertapenem		< 0.5 (100)	< 0.5 (100)	< 0.5 (100)	
Mecropenem		< 0.25 (100)	< 0.25 (100)	< 0.25 (100)	
Ceftriaxone		≤ 1 (100)	< 1 (100)		
Cefepime		≤ 1 (100)			
Tigecycline	< 0.12 (16.7)	< 0.5 (100)	< 0.5 (100)	< 0.5 (100)	
Antibiotic resistance (CMI)					
Clindamicine	≥ 8 (100)				
Peniciline	> 32 (100)				
Tetraciline	> 16 (50)				
Ampiciline		> 32 (66.7)	> 32 (100)	> 32 (100)	
Ciprofloxacine		≥ 4 (100)			
Nitrofurantoine		≥ 4 (100)			
SMZ-TMP					> 32 (50)

increasing to nearly 30% when the risk increases, as it was identified in our study[17,18].

The average age of the patients in whom bactibilia was identified (62 years) is also coincident with the demographic data reported in the literature, that is, after the seventh decade of life[10].

Other important risk factors for the development of a local post-operative infection, such as impaired immune defense, post-operative bleeding, contamination of the abdominal cavity or subcutaneous tissue, or extended operating time, were not present in this cohort.

It is very important to know both the usual pathogenic flora and the bacterial resistance to antibiotics in order to be able to select the most appropriate antimicrobial therapy to be used before a surgical procedure of this nature, thus optimizing the use of available resources[18,19]. In this study, the main sensitivity was found to quinolones and carbapenems, so the first ones should be considered a good option for prophylaxis in cases where acute biliary pathology is identified in the emergency department.

The Tokyo guidelines for antimicrobial management of gallbladder pathology recommend its use according to the degree of severity, so it is important to consider the age, type of pathology and the usual flora coupled with the sensitivity and resistance identified in each institution, under the process called de-scaling, which consists of starting with empirical therapy until obtaining the identification of the causative germ, as well as

the sensitivity and antimicrobial resistance of the causative agent, and then adjusting the antibiotic treatment[5].

In conclusion, following the recommendations of the TG18, it is recommended that in cases of acute biliary pathology in patients who are above the seventh decade of life, the empirical administration of antibiotics of the quinolone group be initiated, and bile culture should be taken before starting gallbladder dissection, for later adjustment of antibiotic therapy according to the results of the culture as needed.

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Conflicts of interest

The authors declare no conflicts of interest.

Ethical considerations

Protection of humans and animals. The authors declare that the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the World Medical Association and the Declaration of Helsinki. The procedures were authorized by the Institutional Ethics Committee.

Confidentiality, informed consent, and ethical approval. The authors have followed their institution's confidentiality protocols, obtained informed consent from all patients, and secured approval from the Ethics Committee. SAGER guidelines have been followed as applicable to the nature of the study.

Declaration on the use of artificial intelligence. The authors declare that no generative artificial intelligence was used in the writing or creation of the content of this manuscript.

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