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Prevalence of and Risk Factors for Biliary Carriage of Bacteria Showing Worrisome and Unexpected Resistance Traits

E. Maseda,[a] G. Maggi,[a] R. Gomez-Gil,[b] G. Ruiz,[b] R. Madero,[c] A. Garcia-Perea,[b] L. Aguilar,[d] F. Gilsanz,[a] J. Rodriguez-Baño[e]

Anesthesiology and Surgical Critical Care Department, Hospital La Paz, Madrid, Spain[a]; Microbiology Department, Hospital La Paz, Madrid, Spain University Complutense, Madrid, Spain[b]; Research Department–Biostatistic Unit, Hospital La Paz, Madrid, Spain[c]; Microbiology Department, School of Medicine, University Complutense, Madrid, Spain[d]; Infectious Diseases and Clinical Microbiology Unit, Hospital University Virgen Macarena, Sevilla, Spain[e]

Data on biliary carriage of bacteria and, specifically, of bacteria with worrisome and unexpected resistance traits (URB) are lacking. A prospective study (April 2010 to December 2011) was performed that included all patients admitted for <48 h for elective laparoscopic cholecystectomy in a Spanish hospital. Bile samples were cultured and epidemiological/clinical data recorded. Logistic regression models (stepwise) were performed using bactobilia or bactobilia by URB as dependent variables. Models ($P < 0.001$) showing the highest R^2 values were considered. A total of 198 patients (40.4% males; age, 55.3 ± 17.3 years) were included. Bactobilia was found in 44 of them (22.2%). The presence of bactobilia was associated (R^2 Cox, 0.30) with previous biliary endoscopic retrograde cholangiopancreatography (ERCP) (odds ratio [OR], 8.95; 95% confidence interval [CI], 2.96 to 27.06; $P < 0.001$), previous admission (OR, 2.82; 95% CI, 1.10 to 7.24; $P = 0.031$), and age (OR, 1.09 per year; 95% CI, 1.05 to 1.12; $P < 0.001$). Ten out of the 44 (22.7%) patients with bactobilia carried URB: 1 *Escherichia coli* isolate (CTX-M), 1 *Klebsiella pneumoniae* isolate (OXA-48), 3 high-level gentamicin-resistant enterococci, 1 vancomycin-resistant *Enterococcus* isolate, 3 *Enterobacter cloacae* strains, and 1 imipenem-resistant *Pseudomonas aeruginosa* strain. Bactobilia by URB (versus those by non-URB) was only associated (R^2 Cox, 0.19) with previous ERCP (OR, 11.11; 95% CI, 1.98 to 62.47; $P = 0.006$). For analyses of patients with bactobilia by URB versus the remaining patients, previous ERCP (OR, 35.284; 95% CI, 5.320 to 234.016; $P < 0.001$), previous intake of antibiotics (OR, 7.200; 95% CI, 0.962 to 53.906; $P = 0.050$), and age (OR, 1.113 per year of age; 95% CI, 1.028 to 1.206; $P = 0.009$) were associated with bactobilia by URB (R^2 Cox, 0.19; $P < 0.001$). Previous antibiotic exposure (in addition to age and previous ERCP) was a risk driver for bactobilia by URB. This may have implications in prophylactic/therapeutic measures.

Bacteria may invade the biliary tract by ascending from the duodenum and by a hematogenous route from the hepatic portal vein. Bactobilia are not found in healthy individuals, since daily excretion of bile helps to flush out whatever organisms enter the biliary tract, but the percentage of bactobilia increases to 3% in patients with gallstones and to ≈30% in patients with common duct stones (1, 2).

About 15% of patients with gallstones are subjected to surgery for uncomplicated symptomatic gallstones (3). The presence of bacteria has been reported in 20 to 40% of patients with symptomatic gallstones but no evidence of cholecystitis (1, 4, 5). There are conflicting reports on the association of positive bile cultures with surgical infections and septic complications (1, 6–9). Postoperative wound infection after elective cholecystectomy ranges from 7 to 20% (6, 10, 11), and the potential benefit of antibiotics is not to eradicate organisms but to control extraluminal extension of infection (12) and to prevent surgical complications. Identification of risk factors for bactobilia and bile cultures during biliary surgery are useful for planning antibiotic prophylaxis and treatment measures (6, 9, 13). However, a recent systematic review of the Cochrane database showed that there is not sufficient evidence to support or refute the use of antibiotic prophylaxis to reduce surgical site infection and global infections in patients undergoing elective laparoscopic cholecystectomy with low risk of anesthetic complications, comorbidities, conversion to open surgery, and infectious complications (14).

The most common isolate from bile at cholecystectomy from patients with uncomplicated cholelithiasis is *Escherichia coli*, followed by *Klebsiella* spp. and *Enterococcus* spp. (6, 11). Although there is an increasing number of reports of worrisome resistance traits (extended-spectrum β-lactamase [ESBL] or AmpC production, vancomycin resistance, methicillin resistance, etc.) for isolates from community-acquired infections (15–19), this has not been reported for biliary isolates from asymptomatic patients with gallstones.

This study aims to explore bile carriage of bacteria with worrisome and unexpected resistance traits and to determine associated risk factors in patients admitted to hospital for <48 h for elective laparoscopic cholecystectomy.

(Part of this study was presented at the 52nd Interscience Conference on Antimicrobial Agents and Chemotherapy, 9 to 12 September 2012, San Francisco, CA [20].)

MATERIALS AND METHODS

A prospective, observational study was carried out from April 2010 to December 2011 in all patients who were >14 years of age and had been admitted for <48 h in Hospital Universitario La Paz (Madrid, Spain) for elective laparoscopic cholecystectomy. A single perioperative dose of amoxicillin-clavulanic acid or cefazolin was used as prophylaxis according to the hospital's routine. Patients hospitalized for >48 h and those

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Address correspondence to E. Maseda, emilio.maseda@gmail.com.

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TABLE 1 Variables showing significance in the three bivariate analyses performed

Parameter	Total	Result according to patient status				
		Sterile bile	Bactobilia	Non-URB bactobilia	URB bactobilia	Sterile bile + non-URB bactobilia
n	198	154	44	34	10	188
Age (yr; means ± SD)	55.3 ± 17.3	51.2 ± 16.3	69.6 ± 12.5[a]	67.6 ± 13.0	76.2 ± 8.2[b]	54.2 ± 17.0[c]
Male (%)	40.4	36.4	54.4[a]	55.9	50.0	39.9
High blood pressure (%)	35.8	29.2	59.1[a]	52.9	80.0	33.5[c]
Stroke (%)	10.7	7.8	20.5[a]	20.6	20.0	10.2
Charlson index (means ± SD)	1.40 ± 0.95	0.82 ± 1.41	1.41 ± 1.28[a]	1.38 ± 1.23	1.50 ± 1.51	0.93 ± 1.40
Proton pump inhibitors (%)	40.4	34.4	61.4[a]	58.8	70.0	38.8
Previous antibiotics (%)	33.8	28.6	52.3[a]	44.1	80.0	31.4[c]
Previous admission (%)	56.1	48.7	81.8[a]	79.4	90.0	54.3[c]
Previous surgery (%)	3.5	2.6	6.8	2.9	20.0	2.7[c]
ERCP (%)	13.6	6.5	38.6[a]	26.5	80.0[b]	10.0[c]
Other endoscopic procedures (%)	18.7	14.9	31.8[a]	32.4	30.0	18.1

[a] $P < 0.05$ for bactobilia versus sterile bile.
[b] $P < 0.05$ for URB bactobilia versus non-URB bactobilia.
[c] $P < 0.05$ for sterile bile plus non-URB bactobilia versus URB bactobilia.

with symptomatic cholecystitis or choleangitis were excluded. The study protocol was approved by the Ethical Review Board of Hospital La Paz (registration no. HULP: PI-1021), and patients signed a written informed consent prior to study inclusion.

Demographic and clinical data were recorded from clinical records, and the age-unadjusted Charlson index (21) (age was considered separately) and the modified McCabe score (Sabadell score) (22) were calculated. Antibiotic intake in the previous 2 months or in the previous year (if more than two antibiotic courses), hospitalizations in the previous 12 months, intake of steroids, history of recurrent urinary tract infections, and the intake of proton pump inhibitors or other antacids within the previous 2 months were recorded. Surgery, biliary endoscopic retrograde cholangiopancreatography (ERCP), or other endoscopic or invasive procedures within the previous 12 months were also recorded.

Bile samples were collected during cholecystectomy and immediately sent to the Microbiology Department for aerobic and anaerobic culture. Identification was performed using Wider (Soria Melguizo S.A., Madrid, Spain) and/or matrix-assisted laser desorption ionization–time-of-flight (MALDI-TOF; Bruker Daltonics, Inc., Billerica, MA) systems. Antibiotic susceptibility was determined using the Wider automated system according to CLSI guidelines (23). ESBLs and carbapenemases were phenotypically confirmed (Etest ESBL and modified Hodge test, respectively). PCRs with specific primers were used for the detection of carbapenemase genes (KPC, VIM, IMP, NDM-1, and OXA-48) and ESBL genes (TEM, SHV, CTX-M, and OXA-1) (24). Vancomycin-resistant or high-level gentamicin-resistant *Enterococcus* spp., methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*, or ESBL- or AmpC-producing enterobacteria were considered bacteria with worrisome and unexpected resistance traits in the community (unexpected resistant bacteria, or URB).

Comparison of proportions was performed by chi-square test and Fisher's exact test when necessary. For quantitative variables, since data did not show normality in the Kolmogorov-Smirnoff test, the Kruskal-Wallis and Mann-Whitney tests were used when necessary. Bivariate analyses were performed to compare all variables between patients with positive culture to those of patients with negative cultures, between patients with URB bactobilia and patients with non-URB bactobilia, and between patients with URB bactobilia and the remaining patients (patients with negative culture plus those with non-URB-positive cultures). Logistic regression models (stepwise procedure) were performed using bactobilia or URB bactobilia as dependent variables and those showing differences ($P \leq 0.1$) in bivariate analyses as independent variables. Interactions and linear dependence between independent variables were previously controlled. The statistical analysis was performed using SPSS, version 4 (SPSS Inc., Chicago, IL). The models showing the highest R^2 values were considered.

RESULTS

A total of 198 patients were included; 44 (22.2%) of them had positive bile cultures. All positive cultures yielded growth of facultative microorganisms, with only 4 out of the 44 (9.1%) in mixed cultures with strict anaerobes (2 patients showing *Clostridium* spp., 1 patient showing *Bacteroides fragilis*, and 1 patient showing *B. fragilis* and *Clostridium* spp.). The most frequently isolated facultative microorganism was *Enterococcus* spp., which was present in 16 out of the 44 (36.7%) patients with bactobilia, followed by *E. coli* and *Klebsiella* spp., present in 13 (29.5%) patients each. Ten patients presented URB isolates (10/44; 22.7%): 3 patients with *Enterobacter cloacae*, 2 with ESBL-producing enterobacteria (1 CTX-M-producing *E. coli* and 1 OXA-48-producing *K. pneumoniae*), 2 with high-level gentamicin-resistant *Enterococcus faecium*, 2 with vancomycin-resistant *Enterococcus casseliflavus* (with one of them also showing high-level gentamicin-resistant *Enterococcus faecalis*), and 1 with a *P. aeruginosa* isolate resistant to piperacillin-tazobactam, ceftazidime, cefepime, and imipenem.

Table 1 includes variables showing significance in any of the three bivariate analyses performed. The group of patients with bactobilia (compared to those with negative culture) had a significantly ($P < 0.05$) higher mean age and Charlson index. This group also showed significantly higher percentages of males and of patients with high blood pressure, with previous stroke, with hospitalization in the previous year, with intake of proton-pump inhibitors and of previous antibiotics, and with previous ERCP or other endoscopic procedures than patients without bactobilia. In the multivariate analysis (R^2 Cox, 0.297; $P < 0.001$), previous ERCP (odds ratio [OR], 8.951; 95% confidence interval [CI], 2.961 to 27.056; $P < 0.001$), previous admission (OR, 2.821; 95% CI, 1.099 to 7.240; $P = 0.031$), and age (OR, 1.081 per year of age; 95% CI, 1.048 to 1.116; $P < 0.001$) were significantly associated with bactobilia.

The group of patients with URB bactobilia (compared to those with non-URB bactobilia) had a significantly ($P < 0.05$) higher

mean age and a higher rate of patients with previous ERCP (Table 1). In the multivariate analysis (R^2 Cox, 0.192; $P < 0.001$), only previous ERCP (OR, 11.110; 95% CI, 1.976 to 62.466; $P = 0.006$) was associated with URB bactobilia when the analysis was adjusted by age and by previous intake of antibiotics.

In comparisons of the group of patients with URB bactobilia to grouped patients showing no bactobilia or non-URB bactobilia (Table 1), significantly ($P < 0.05$) higher mean ages and higher percentages of patients with high blood pressure, with previous intake of antibiotics, with previous surgery, with hospitalization in the previous year, and with previous ERCP were found among patients with URB bactobilia. In the multivariate analysis (R^2 Cox, 0.194; $P < 0.001$), previous ERCP (OR, 35.284; 95% CI, 5.320 to 234.016; $P < 0.001$), previous intake of antibiotics (OR, 7.200; 95% CI, 0.962 to 53.906; $P = 0.050$), and age (OR, 1.113 per year of age; 95% CI, 1.028 to 1.206; $P = 0.009$) were associated with URB bactobilia.

DISCUSSION

Some reports have shown higher incidences of postoperative morbidity and infectious complications in patients with bactobilia versus those without it (2, 25). It is well known that bactobilia is a common finding in patients with risk factors, such as biliary obstruction, age of >70 years, acute cholecystitis, common bile duct stones, cholangitis, and nonfunctioning gallbladders (13, 26). In patients undergoing laparoscopic cholecystectomy, a previous study showed that preoperative ERCP and age were predictors of bactobilia (7). It has been suggested that prophylactic antibiotics should be limited to patients with risk factors of bactobilia (13), but there is not sufficient evidence to support or refute the use of antibiotic prophylaxis in patients undergoing elective laparoscopic cholecystectomy with low risk of complications (14). Since bactobilia status at the time of surgery, and especially in the presence of resistant bacteria, is unknown, identification of predictors of bactobilia by resistant bacteria is important. In this sense, as in previous publications on intra-abdominal infections (27, 28), the concept of resistant bacteria that we used in the present study was wider than the classical definition of multidrug-resistant bacteria (i.e., acquired resistance to three or more antimicrobial classes) to include vancomycin-resistant and high-level gentamicin-resistant *Enterococcus* spp.; methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*; and ESBL- and AmpC-producing enterobacteria (all of them are considered URB in bile).

In the present study, bactobilia was found in 22.2% of patients with elective laparoscopic cholecystectomy, with predictors of bactobilia being previous ERCP, previous hospital admission, and age, as described in a previous report (7). More interestingly, 22.7% of those with bactobilia presented URB isolates. This fact should be considered in the selection of the antibiotic used for prophylaxis for cholecystectomy in patients with risk factors. Three *E. cloacae* isolates (that must be considered inducible AmpC chromosomal β-lactamase producers [15]), one ESBL-producing *E. coli* strain (thus conferring resistance to narrow-, expanded-, and broad-spectrum cephalosporins and aztreonam [29]), and one carbapenemase-producing *K. pneumoniae* strain (conferring resistance to all current β-lactams) were isolated in addition to a *P. aeruginosa* isolate that also was resistant to cephalosporins and carbapenems. With respect to Gram positives, four *Enterococcus* spp. (two with vancomycin resistance and two with aminoglycoside high-level resistance) were isolated. In enterococci, in addition to the acquisition of resistance to ampicillin, aminoglycoside (high-level) and glycopeptide resistance is a cause for concern (17).

Risk factors identified in the multivariate analysis for the isolation of these URB bacteria were previous ERCP and age, as for bactobilia, but importantly, they also included previous antibiotic exposure (OR of 7.200 versus patients with non-URB bactobilia). Unfortunately, since only 10 URB isolates were identified, no association could be found with antibiotic classes previously taken by these patients. The results of the present study warrant wider studies to explore the relationship between URB bactobilia and postsurgery complications.

In conclusion, the prevalence of bactobilia found in the community of patients undergoing elective laparoscopic cholecystectomy (with nearly one-fourth of patients with bactobilia carrying URB bacteria) was not negligible. When establishing prophylactic or therapeutic measures in patients presenting risk factors for bactobilia, it should be taken into account that previous antibiotic exposure is a risk driver for bactobilia by bacteria with worrisome and unexpected resistance traits.

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