BMJ Open

Predictive factors associated with Bile culture positivity And phenotypiCal antIbiogram resistance patterns in patients taken to LaparOscopic cholecystectomy (BACILO): protocol for a prospective observational cohort study and development of a prognostic prediction model

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To cite: Ramírez-Giraldo C, Rodriguez Barbosa C, Isaza-Restrepo A, *et al*. Predictive factors associated with Bile culture positivity And phenotypiCal antIbiogram resistance patterns in patients taken to LaparOscopic cholecystectomy (BACILO): protocol for a prospective observational cohort study and development of a prognostic prediction model. *BMJ Open* 2024;**14**:e086655. doi:10.1136/bmjopen-2024-086655

► Prepublication history for this paper is available online. To view these files, please visit the journal online (https://doi.org/10.1136/bmjopen-2024-086655).

Received 19 March 2024
Accepted 30 September 2024

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ABSTRACT

Introduction Bile fluid is aseptic under normal conditions; however, in the presence of cholecystitis, its susceptibility to bacterial colonisation varies, with reported rates of 20%–70% of cases. This process is referred to as bactibilia and/or bacteriobilia and can be considered a secondary complication of biliary stasis and cholecystitis in general. In the management of acute cholecystitis, the antibiotic regimen should be prescribed based on the presumed pathogens involved, taking into consideration the risk factors for resistance patterns according to demographics and local exposure. The aim of this study is to determine the predictive factors for bile culture positivity and antibiotic resistance in patients who underwent laparoscopic cholecystectomy in the Méderi Hospital Network. We hope to develop a predictive model that allows us to better guide antibiotic therapy.

Methods and analysis This is a prospective observational cohort study with prognostic prediction model. Patients who will undergo laparoscopic cholecystectomy and have bile cultures taken in the Méderi Hospital Network during the study period will be included. The dependent variables will be positive bile culture and antibiotic resistance, and the predictive variables will be age, presence of diabetes, diagnosis of choledocholithiasis, diagnosis of cholecystitis and severity of cholecystitis according to the Tokyo criteria. The minimum sample size has been calculated at 703 patients. Follow-up will continue until a control appointment 15 days after the procedure. The primary outcomes are bile culture positivity and phenotypical antibiogram resistance. For each outcome, a multivariate logistic regression will be performed using frequentist and Bayesian prediction techniques.

Ethics and dissemination This study was approved by the Méderi network research department committee (CIMED) and by Universidad del Rosario's Research Ethics Committee (CEI-UR; DVO005 2555-CV1837). Written informed consent is required for participation. The results will be disseminated through the submission of an academic article to a high-impact scientific journal, presentations at academic conferences, and sharing with our institution's faculty to inform antimicrobial therapy management based on local epidemiological data.

Trial registration number NCT06314399.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This is a prospective study and the variables included have been previously established based on current scientific literature.
⇒ Data should be representative of the target population and application setting.
⇒ The sample size was established considering the development of and internal validation of the model.
⇒ The study is based in a single centre, which does not allow evaluation of differences in microbial patterns between different institutions region-wide and reduces the generalisability of the results.

INTRODUCTION

Under usual circumstances, bile is an antiseptic fluid; however, in patients with cholecystitis, there is an increased and variable susceptibility to bacterial colonization, ranging from 20% to 70% of cases. This process is denominated as bactibilia and/or bacteriobilia and can be considered a secondary complication of biliary stasis and consequently cholecystitis.[1]

Definitive treatment for this condition must be multidisciplinary and includes antibiotic therapy, analgesic management or, if the case requires, use of intravenous fluids with posterior surgical intervention.It is also important to establish that, although surgical management is a fundamental pillar of treatment, initiating early antibiotic therapy targeting the most common micro-organisms significantly reduces the risk of sepsis. This is the reason why the World Society of Emergency Surgery (WSES) recommends an antibiotic regimen based on the most allegedly involved pathogens as standard treatment for acute cholecystitis, taking into account the risk factors for phenotypical resistance patterns according to local exposure and demographics. Considering all the aforementioned, it is key to know the microbial spectrum associated with acute cholecystitis in our population so as not to fall prey to suboptimal treatment regimens and to help dissipate the morbimortality curve at our institution.[2] Thus, the objective of this study is to identify the predictive factors associated with positive bile culture and phenotypical antibiogram resistance patterns in patients taken for laparoscopic cholecystectomy in the Méderi Hospital Network.

METHODS AND ANALYSIS

Objectives of the study

The primary objective of this study is to determine which predictive factors are associated with positive bile culture and phenotypical antibiogram resistance patterns in patients taken for laparoscopic cholecystectomy in the Méderi Hospital Network.

The following are the secondary objectives:

- Describe and compare the demographic, clinical and surgical characteristics, as well as surgical outcomes in patients with positive bile cultures and phenotypical antibiogram resistance versus patients with negative bile culture and phenotypical antibiogram sensitivity.
- Determine the demographic and clinical predictive factors for positive bile cultures.
- Determine the demographic and clinical predictive factors for phenotypical antibiogram resistance.
- Explore the relationship between positive bile cultures and phenotypical antibiogram resistance with surgical outcomes.
- Explore the correlation between the micro-organism isolated in bile cultures and the micro-organism isolated in a surgical site infection (SSI; if applicable and if the patient presents it).
- Perform internal validation for prognostic study models.

Study design

This is a single-centre, observational, analytical, prospective cohort study with a prognostic prediction model[3] and will be reported following ‘the TRIPOD+AI statement: updated guidance for reporting clinical prediction models that use regression or machine learning methods’[4] (online supplemental file 1).

Study setting

This study will be carried out in the Méderi Hospital Network in Bogotá, Colombia. It is a single-centre study in two different institutions operating under the same network: Hospital Universitario Mayor and Hospital Universitario Barrios Unidos.

Patient and public involvement

The patients were not included in the design of the research. When the study is published, the results will be reported in a simple and clear manner so that patients who wish to know the results can do so.

Inclusion and exclusion criteria

Patients included must be over 18 years of age and be taken for laparoscopic cholecystectomy at any institution of the Méderi Hospital Network (Hospital Universitario Mayor and Hospital Universitario Barrios Unidos), have signed the written informed consent and have both bile culture and antibiogram.

Patients meeting any of the following criteria will be excluded:

- Patients who will be taken for laparoscopic cholecystectomy with another concomitant surgical procedure (including but not exclusive to gastrectomy, pancreatoduodenectomy, oesophagectomy, splenectomy, abdominal wall reconstruction and colectomy, among others), with the exception of umbilical herniorrhaphy.
- Patients without postoperative control appointment.
- Patients with a previously documented diagnosis of gallbladder or biliary tract malignant disease.

Sample size

In identifying the predictive factors for positive bile culture and phenotypical antibiogram resistance, the minimum number of patients needed to achieve statistical significance is 703. This was determined using data from Lee *et al*[5] as reference, which reported a value of 52.12% for positive bile cultures and 25.25% for antibiotic resistance. Bearing these values in mind, the sample was calculated using a model for prognostic predictive studies with a total of seven predictive factors (table 1). This was calculated with Stata V.17 using the *pmsampsize* command.

The sample size was adjusted to account for a potential 5% loss, and an additional calculation was performed to include 15% for internal model validation, resulting in 703 participants as the significant number for both primary outcomes (table 2).

Outcomes

The main outcome of this study is a reliable information on the local epidemiology of bacterial colonisation in bile fluid of patients taken for laparoscopic cholecystectomy

Table 1 Predictive factors included in sample size calculation

Variables	Type/category	Number of parameters
Age	Continuous	1
Diabetes mellitus	Yes/no	1
Choledocholithiasis: ERCP	Yes/no	1
Cholecystitis	Yes/no	1
C reactive protein	Continuous	1
Severity of cholecystitis according to 2018 Tokyo guidelines		
Tokyo I		2
Tokyo II		
Tokyo III		
Total		7

ERCP, endoscopic retrograde cholangiopancreatography.

in the Méderi Hospital Network in Bogotá, Colombia, specifically the rate of patients with positive bile cultures and the microbial typification and antibiotic resistance in these cultures, as well as the risk factors that predispose patients to it. This will be represented in the following manner:

- The primary outcomes are bile culture positivity and phenotypic antibiotic resistance patterns, with six variables considered as potential predictive factors: age, presence of diabetes mellitus diagnosis, concomitant choledocholithiasis or previous endoscopic retrograde cholangiopancreatography (ERCP), C reactive protein values, and presence of cholecystitis and the severity according to the 2018 Tokyo guidelines.
- The secondary outcomes to be measured include a description of demographic, surgical and clinical characteristics, as well as surgical outcomes (complications according to the Clavien-Dindo score, intensive care unit admittance and stay, and hospital stay length), in the sample and a comparison between the groups presenting bile culture positivity versus negativity and antibiotic resistance versus sensitivity.

Bile cultures and antibiotic susceptibility testing

Bile culture will be taken intraoperatively from the surgical specimen, labelled anonymously and sent for analyses in the institutional laboratory. The bile sample will be obtained by puncturing the gallbladder wall with a #14 French catheter through the skin. After obtaining the sample, the puncture orifice will be occluded with a grasper forceps to avoid uncontrolled bile spillage. In case of bile spillage, the abdominal cavity will be adequately cleaned.

The bile samples will be inoculated on agar plates using MacConkey, thioglycolate and both microaerophilic and GasPak anaerobic chocolate agar plates. They will then be incubated for 24, 48 and 72 hours in an aerobic environment and up to 240 hours in an anaerobic environment. For positive culture, the antibiogram will be processed through the Phoenix M50 system, and carbapenemic resistance will be detected through PCR. If any yeast growth is detected, the agar will be sent to a reference laboratory, where it will be processed through a microdilution technique.

Data collection

Data collection has not begun but is expected to begin in April 2024. Data will be collected and stored online using the Research Electronic Data Capture (REDCap) web application server, which allows collaborators to store data securely as it does not allow third-party access, with the intent to protect patient data. For both the initial basal recollection and the first postoperative follow-up appointment, both data recollection and storage will be a task of the general surgery resident on shift (this is part of the integral care model at our institution for all patients in whom a surgical procedure is performed). Each collaborator will be assigned personal login data in the REDCap system so that these are sent securely to the server. Only anonymous data, without any individual patient identifiers, will be uploaded to the database.

All patients who meet the inclusion criteria will be included in the study, with demographic, clinical, and surgical variables collected at the time of hospital admission. They will be followed up with a control appointment 15 days after laparoscopic cholecystectomy, conducted by the research group

Once the study is finalised, all data will be stored in Méderi Corporation's servers for a period of 15 years, complying with Resolution 839 of 2017. All data collected will be consolidated into an anonymous database without any individual or identifying characteristics, maintained by the Centre for Learning and Research Resources (CRAI) at Universidad del Rosario. If these data are considered useful for a posterior analysis, a new research protocol must be submitted to and approved by both the Méderi network research department committee (CIMED) and Universidad del Rosario's Research Ethics Committee (CEI-UR).

Table 2 Sample size calculation process

Outcome	Number of parameters	%	R-squared	Variability (%)	Total (n)
Positive culture: yes/no	7	52.12	0.112	15	636
Resistance: yes/no	7	25.25	0.102	15	703

Information quality control

Patient data will be collected from electronic medical records in a database. This will comply with confidentiality rules, with the institution or the researcher excluding any possible identifiers. To ensure quality of information and minimise risk of information bias, collaborators tasked with data collection will receive specific training. Clinical variables will be directly taken from the institution's primary records of clinical history.

The research group is committed to submitting periodic reports on partial results and on completion will present a final report to CIMED in order to establish a continuous follow-up of the research process. These reports will include all databases and will be archived without any identifier or variable that could identify or establish communication with any of the research subjects subject to clinical history review.

Data analysis plan

Descriptive analysis of the data will be performed to determine the outcomes for both primary and secondary objectives. Categorical variables will be described as absolute and relative frequencies (percentages). Continuous variables will be evaluated according to their distribution using the Shapiro-Wilk statistical test and will be presented as mean and SD (if they present a normal distribution) and as median and IQR (when they present a non-normal distribution) for each of the analysed groups (positive bile cultures and phenotypical antibiogram resistance vs negative cultures and antibiotic sensitivity).

Multivariate logistical regression will be performed for each outcome using frequentist and Bayesian techniques, taking positive bile culture and phenotypical antibiogram resistance as the dependent variables, and using age, presence of diabetes, diagnosis of choledocholithiasis and/or cholecystitis and severity of cholecystitis according to Tokyo guidelines as the predictive variables. For the frequentist analysis, a stepwise method will be performed to choose predictive variables, establishing a p value <0.05 as statistically significant. For the Bayesian analysis, probability distributions will be initially assigned to the independent variables and to the coefficients of the predictive variables. Through a posterior simulation using Markov chains, an estimated coefficient estimate will be analysed to determine the direction between the relationship in the included variables and to then determine the predictive variables using a credibility interval interpretation. Furthermore, a diagnostic process will be performed on the models to evaluate convergence between distributions.

Model calibration and discrimination will be performed using 15% of the calculated sample. The discriminative ability of the model will be evaluated using the apparent and adjusted C-statistic. Calibration will be reported using the Hosmer-Lemeshow test and shrinkage factor.

All statistical analyses will be performed using RStudio and Stata V.17.

Ethics and dissemination

This study was approved by CIMED and CEI-UR under approval number DVO005 2555-CV1837. This study will comply with the Health Ministry's Resolution 8430 of 1993 on scientific, technical and administrative policies on research in the healthcare sector. This study is classified as having minimal risk considering it is a prospective study that uses history records and direct recollection of primary data from the patients, with extraction of bile during cholecystectomy for cultures and antibiogram.

Ethical principles and guidelines established by the Belmont Report for the protection of human research subjects will be followed, respecting all persons and their autonomy, beneficence and justice. All data collected will undergo anonymisation and will only be input into the database once written informed consent form is signed by the patient or the patient's guardian.

The main researcher will guarantee that all responsibilities during the research will be adhered to, including the inclusion and exclusion criteria and proper implementation of the methods relevant to the research process, while documenting and structuring valid, trustworthy results. The main researcher is also tasked with reporting any results or interpretations to CIMED and/or CEI-UR, be it positive or negative results. Any change or breach of this condition will be reported anonymously to the chief of the surgical department where the study is being performed and to CIMED and/or CEI-UR.

Dissemination is planned to occur in the following scenarios: by submission and publication of the results in the form of an academic article in a scientific journal on general surgery, by presentation of the results to academic and/or scientific events on general surgery and by submitting the results for evaluation by professionals in general surgery and infectiology at our institution to determine possible changes in clinical conduct on the ideal start and choice of antibiotic therapy for patients admitted to the institution, according to locally reported epidemiological data.

DISCUSSION

The BACILO (Predictive factors associated with Bile culture positivity And phenotypiCal antIbiogram resistance patterns in patients taken to LaparOscopic cholecystectomy) study was designed with the objective of obtaining robust data on local epidemiological bacterial colonisation in bile cultures of patients taken for laparoscopic cholecystectomy at our institution and to identify which predictive factors are associated with culture positivity and antibiotic resistance patterns, with the interest of ensuring better decision-making in terms of targeted treatment based on local exposure and scientific evidence.

Although the pathogens responsible for biliary infection have been amply characterised in a wide number of studies, it is safe to affirm that the principal cause is of bacterial aetiology, with consideration for fungal infections (particularly of *Candida* and *Aspergillus* species) in special populations such as in immunosuppressed patients. Viral infections tend to be very infrequently associated with this pathology.[6] Furthermore, the presence of bactibilia has been associated with higher morbidity rates of total as well as infectious and surgical complications, including longer hospital stays and the need for antibiotics.[7 8]

Being acquainted with the most common pathogens responsible for biliary infections has allowed the discernment of superior treatment strategies. The commonly isolated bacteria are usually Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter freundii*) and Gram-positive *Enterococcus* spp.[9–11]

However, after publication of the 2018 Tokyo guidelines, microbiological patterns have changed as bacteria acquire resistance patterns to empirical antibiotic therapy administered by personnel of the healthcare sector.[11] Mamatha *et al*[6] published a study in 2019 on patients diagnosed with biliary tract infections admitted to a third-level health centre in Manipal where both bacterial and fungal aetiologies were registered alongside their antimicrobial resistance pattern. The study evidenced that, out of 307 bile cultures sent for aerobic cultures and susceptibility tests, 187 (60.91%) had positive cultures, with *E. coli* (44.4%) being the most predominant micro-organism, followed by *K. pneumoniae* (27.3%). Anaerobic agents were identified in five (35.75%) cultures, with *Bacteroides fragilis* being the most frequent. Of 201 bacterial pathogens tested for antimicrobial susceptibility, 108 (53.73%) were isolated as multidrug-resistant. All *Candida* species were sensitive to fluconazole therapy, except for *C. glabrata* and *C. krusei*. All anaerobic pathogens were sensitive to metronidazole, which is expected considering that these two micro-organisms' typical resistance patterns include resistance to azoles.[6]

The WSES reports a similar tendency to those described in the Tokyo guidelines, highlighting that Gram-negative aerobics *E. coli* and *Klebsiella*, alongside anaerobic *B. fragilis*, are the most frequent pathogens responsible for biliary infections. Although their pathogenicity is not clearly understood, much of the literature before this study registered extended-spectrum beta-lactamase Enterobacteriaceae as the main mechanism, mainly in patients with community-acquired infections who had a previous exposure to antibiotic therapy.[2]

In addition to this, Wu *et al* performed a study on the distribution and changes in antibiotic resistance in patients with acute infections of the biliary tract in a large series of patients taken for either drainage by gallbladder puncture or ERCP, with the extraction of 3–5 mL of bile for bacterial cultures and identification and antibiotic sensitivity. Due to the study's chronicity, the sample was divided into two cohorts, defined by hospital admittance before and after 2014. A total of 376 bacterial strains were found, of which 98 (26.1%) were Gram-positive, 269 (71.5%) were Gram-negative and 9 (2.4%) were fungi. The three main Gram-positive bacteria were *Enterococcus faecium* (48/98, 49%), *Enterococcus faecalis* (20/98, 20.4%) and *Enterococcus luteus* (7/98, 7.1%). The five most common Gram-negative bacteria were *E. coli* (90/269, 33.5%), *Klebsiella pneumoniae* (37/269, 13.8%), *Pseudomonas aeruginosa* (35/269, 13%), *Acinetobacter baumannii* (34/269, 12.6%) and *Enterobacter cloacae* (13/269, 4.8%), concluding that the most common pathogens responsible for acute biliary tract infections were Gram-negative bacteria. From 2009 to 2019, the proportion of different Gram-negative remained consistent, but the rates of antibiotic resistance had incremental tendencies, particularly for *E. coli*.[12]

Manrai *et al*[13] recently performed a microbial characterisation in the Indian population, taking aseptic bile cultures of patients with cholestasis taken for ERCP, with positive cultures being taken for typification and antibiotic sensitivity, registering a predisposition to polymicrobial infections in patients with previous biliary tract instrumentation. Nonetheless, pathogen typification was consistent with what had been previously described in both the 2018 Tokyo and the WSES guidelines. Gram-negative bacteria were predominant, with *E. coli*, *P. aeruginosa* and *K. pneumoniae* being the three most common pathogens, accounting for 70% of the bacterial strains identified. The most common antibiotic sensitivity was to piperacillin-tazobactam and imipenem, while the most commonly used antibiotics had significant sensitivity and can still be used empirically. However, patients with previous implantation of biliary stents can be at a higher risk of Enterococcus spp infection and could consequently require targeted therapy. Some of the most commonly described predictive factors for positive cultures and antibiotic resistance patterns found in the current literature can be seen in tables 3 and 4.

Similar data have been found in our population, with the current literature reporting *E. coli* as the most commonly isolated micro-organism, followed by *Klebsiella*.[14] Nonetheless, antibiotic resistance patterns differ

Table 3 Predictive factors for positive bile cultures

Predictive factor	Positive cultures	Negative cultures
Age[5]	68±12.8	58.5±17.1
Diabetes mellitus[17] (%)	55	45
Choledocholithiasis: ERCP[5] (%)	25.9	8.9
C reactive protein[5]	14.6±17.9	7.9±12.1
Cholecystitis[18] (%)	53.2	39.8
Severity of acute cholecystitis according to 2018 Tokyo guidelines[5]		
Tokyo I (%)	43.97	56.02
Tokyo II (%)	63.80	36.19
Tokyo III (%)	63.63	36.36

ERCP, endoscopic retrograde cholangiopancreatography.

Table 4 Predictive factors for antibiotic resistance in positive bile cultures

Predictive factor	Resistant micro-organism	Sensitive micro-organism
Age[5]	71.6±8.4	68.1±12.3
Choledocholithiasis: ERCP[5] (%)	36	6.8
Severity of acute cholecystitis according to 2018 Tokyo guidelines[5]		
Tokyo I (%)	25.53	74.47
Tokyo II (%)	27.08	72.92
Tokyo III (%)	0	100.0
History of antibiotic use 90 days before hospital admittance[19]	Crude OR: 1.48 (95% CI 1.38 to 1.59) Adjusted OR: 1.12 (95% CI 1.03 to 1.21)	

ERCP, endoscopic retrograde cholangiopancreatography.

from what has been currently reported, as most micro-organisms are multisensitive.[15]

Another aspect we would like to explore is the correlation between the micro-organism isolated in bile cultures and those isolated in a surgical site infection (SSI) if the patient presents it, as this would help us target antibiotic therapy for SSI. Sutton *et al*[16] evaluated the correlation between these after pancreatoduodenectomy and reported no correlation between the micro-organisms isolated in bile cultures and in SSI. To our knowledge, an evaluation of this correlation has not been reported in the current literature among patients taken for laparoscopic cholecystectomy.

Some limitations of this study include it being based in a single centre, which does not allow us to evaluate differences in microbial patterns between different institutions region-wide. Nonetheless, we hope to perform external validation of the model to generalise our results to the overall population. Furthermore, our predictive model was designed before collecting and analysing data using variables of interest that have already shown significance in previous literature. This means that even if another variable would have been predictively significant, it would be overlooked as it was not previously included in the predictive model.

Bacteria and antibiotic resistance patterns are areas of medicine that are not only subject to available scientific literature but differ depending on geographical region, time and institution where the patient has been been admitted. There are currently no specific epidemiological data on bile culture characteristics at our institution, thus the necessity to provide better understanding to establish evidence-based and targeted therapy to patients based on our institution's environment. Furthermore, as this is an ever-changing subject, this knowledge would be valuable to other physicians interested in current microbiological trends, as literature on the topic is scarce. The need to provide patients with effective and updated care is crucial to avoiding suboptimal therapy and possible future complications.

STUDY STATUS

This study was registered in ClinicalTrials.gov on March 2024 under registration number NCT06314399. Data collection is expected to start in April 2024 and end in October 2024. Data analysis will be performed between November and December 2024.

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Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

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