

Original Article

Gallbladder microbiota variability in Colombian gallstones patients

Ariel Antonio Arteta¹, Hernán Carvajal-Restrepo², Miryan Margot Sánchez-Jiménez², Sergio Díaz-Rodríguez¹, Nora Cardona-Castro^{1,2}¹ Faculty of Medicine - CES University, Medellín, Colombia² Colombian Institute of Tropical Medicine – CES University, Medellín, Colombia**Abstract**

Introduction: Gallbladder stones are a very frequently occurring condition. Despite bile bactericidal activity, many bacteria have been detected inside the gallbladder, and gallstones facilitate their presence. Between 3% and 5% of the patients with *Salmonella* spp. infection develop the carrier stage, with the bacteria persisting inside the gallbladder, shedding bacteria in their feces without signs of infection. The aim of this study was to isolate bacteria from Colombian patients with gallstones, using standard culturing methods, and to identify *Salmonella* spp. carriers by molecular techniques.

Methodology: A total of 149 patients (120 female and 29 male) diagnosed with gallstones who underwent cholecystectomy and who did not have symptoms of acute inflammation were included. Gallbladder tissue and bile were cultured and used for DNA extraction and *Salmonella* spp. *hila* gene detection.

Results: Of the 149 patients 28 (19%) had positive cultures. Twenty-one (75%) patients with positive cultures were from Medellín's metropolitan area. In this geographical location, the most frequent isolations were *Pseudomonas* spp. (38%), *Klebsiella* spp. (23%), and *Proteus* spp. (9%) in addition to unique cases of other bacteria. In Apartado, the isolates found were *Enterobacter cloacae* (50%), *Raoultella terrigena* (32%), and both *Enterobacter cloacae* and *Raoultella terrigena* were isolated in one (18%) male patient. Five (3.3%) of the 149 patients had positive polymerase chain reaction (PCR) results for the *hila* gene of *Salmonella* spp., all of whom were female and residents of the Medellín metropolitan area.

Conclusions: The gallbladder microbiota variability found could be related to geographical, ethnic, and environmental conditions.

Key words: Gallstones; microbiota; enterobacterium; cholecystitis

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Introduction

Gallbladder stones occur very frequently, with prevalence rates between 10% and 30% in adult populations of developed countries [1]. The pathogenesis of gallstones is a multifactorial phenomenon related to age, ethnicity, diet, gender, body mass index, family history, and metabolic diseases, among other factors [2].

Bacteria have been implicated in the pathogenesis of gallstones, producing high levels of beta-glucuronidase, biliary mud, and phospholipases A2 [3], not only in non-pigmented gallstones where oversaturation of cholesterol prevails, but also in pigmented gallstones where there are high proportions of unconjugated bilirubin [4]. Additionally, gallstones facilitate the presence of bacteria inside the gallbladder, adhering to the stones and making a bacterial community inside a polymeric extracellular matrix called biofilm [5].

Bile is produced by hepatocytes and is stored and concentrated in the gallbladder [6]. Its function is to facilitate the absorption of lipophilic substances such as fats and vitamins [7], to excrete cholesterol, and to regulate the intestinal microbiota through its bactericidal activity [8,9].

Despite bile bactericidal activity, effected by damage to the cellular membrane and to DNA, many bacteria are able to survive inside the gallbladder [10]. This organ is reached by ascending from the duodenum, via the hematogenous route or via enterohepatic circulation [11].

Many bacteria have been detected from the bile using conventional methods (cultures) or molecular techniques, such as the amplification and sequencing of the 16S rRNA gene [12]. Using molecular techniques, a study of Chinese patients with gallstones revealed *Firmicutes* and *Bacteroidetes* as the first and second most abundant phyla, mirroring the major distribution of bacteria in the intestine. Their bile also show

increased relative abundance of phyla such as *Proteobacteria*, *Actinobacteria*, *Thermi*, *Cyanobacteria*, and *Tenericutes* when compared to normal feces [13]. Groups such as *Enterobacteriaceae*, *Ruminococcaceae*, *Bacteroidales*, *Lactococcus*, *Enterococcus*, and *Clostridiales* predominated in the bile of patients with gallstones. *Escherichia coli*, *Klebsiella*, *Enterobacter*, *Acinetobacter*, *Pseudomonas*, and *Citrobacter* were the most frequently occurring species [13,14].

Between 3% and 5% of the patients with *Salmonella* spp. infection develop the carrier stage, in which the bacteria persists inside the gallbladder [15]. Patients who develop the carrier stage can shed bacteria in their feces for months, years, or even the rest of their lives without signs of infection [16]. These individuals are of utmost importance from the perspective of public health organizations as they become reservoirs, and 25% of them do not realize they are infected [17,18].

The isolation of *Salmonella* spp. is a difficult task due to scarce bacteria in the different samples. Due to the low sensitivity of the cultures and the high rate of false-positive results in the serologic test, an alternative approach for the diagnosis, using techniques based on the amplification and detection of nucleic acids, has been suggested [19].

Bacterial communities from different anatomic locations, principally in the gastrointestinal tract, vary in presence and relative abundance of phyla, genera, and species, depending on factors related to geography, population, ethnicity, diet, environment, and sanitation [20,21]. Colombia is an endemic country for *Salmonella* spp. infections [22,23], yet there have been no local surveys of the gallbladder microbiota. The aim of this study was to isolate bacteria from the bile of Colombian patients' gallbladders using standard culturing methods, and to identify *Salmonella* spp. carriers by molecular techniques.

Methodology

Patients

A total of 149 patients diagnosed with gallstones who underwent cholecystectomy were included in the study after informed consent was procured. Of the patients, 13 were from Apartadó (Antioquia), 2 were from Quibdó (Chocó), and 134 were from Medellín's metropolitan area.

Gallbladder and bile samples

Instructions were given to the surgeons in charge of the gallbladder resections in each city. The instructions involved sampling tissue of the gallbladder to be used

in histopathology (10% tamponade formol) for DNA extraction (70% ethanol) and bile in lactose broth for cultures and DNA extraction.

Cultures

Bile samples were collected in lactose broth (BBL, Sparks, USA) for pre-enrichment, incubated at 35°C for 12 hours, and then replicated in blood agar, MacConkey and Salmonella-Shigella (SS) agars (all from BBL, Sparks, USA) and incubated again for 12 hours at 35°C. One milliliter of the previously enriched bile was cultured in selenite broth and incubated again for 12 hours at 35°C, and then replicated in MacConkey and SS agars. Biochemical identification of the isolated colonies was done using the API20E system (bioMérieux, Durham, USA).

DNA extraction

DNeasy Blood and Tissue Kit (QIAGEN, Duesseldorf, Germany) was used for DNA extraction from gallbladder tissue and bile.

Polymerase chain reaction (PCR)

Salmonella spp. *hlyA* gene detection was performed as previously described [24,25]. Briefly, PCR mix comprised 40 µL of SuperMix (Invitrogen, Carlsbad, USA), 0.5 µL of the forward primer at 10 µM, 0.5 µL of the reverse primer at 10 µM, and 9 µL of the DNA, for a total volume of 50 µL at 0.1 µM. The amplification protocols were as follows: initial denaturation at 94°C for 5 minutes, 30 cycles at 94°C for 1 minute, 65°C and 72°C for 1 minute each, and a final elongation phase of 10 minutes at 72°C. The visualization of the 854 base pairs was done using a 1% agarose gel stained with SYBR Safe (Invitrogen, Carlsbad, USA) and revealed in an EpiChemi Darkroom image analyzer (Upland, USA).

Results

Patients

Of the 149 patients involved in the study, 120 were females (80.5%) and 29 were males (19.5%) with a median age of 39 years (range 27–66). Twenty-eight (19%) of them had positive cultures (23 females and 5 males), with one bacterium isolate in 27 patients (97%) and two bacterial isolates only in 1 patient (3%).

Twenty-one (75%) patients with positive cultures were from Medellín's metropolitan area, six (21.5%) of them were from Apartadó (Antioquia), and one (3.5%) was from Quibdó (Chocó). Of the six patients with positive cultures from Apartadó, *Enterobacter cloacae* was isolated in three (50%), *Raoultella terrigena* in two

Table 1. Patients’ origin, bacteria isolations, frequencies, and *hilA* *Salmonella* PCR results.

Origin	Culture result	Isolation	Frequency	Frequency/ <i>hilA</i> PCR
Medellin	Positive	<i>Pseudomonas</i> spp.	4	Negative
		<i>Klebsiella pneumoniae</i>	4	Negative
		<i>Pseudomonas fluorescens/putida</i>	3	1 (one) <i>hilA</i> <i>Salmonella</i> PCR positive patient
		<i>Pseudomonas aeruginosa</i>	1	Negative
		<i>Proteus penneri</i>	1	Negative
		<i>Proteus mirabilis</i>	1	Negative
		<i>Stenotrophomonas maltophilia</i>	1	Negative
		<i>Serratia marcescens</i>	1	Negative
		<i>Escherichia coli</i>	1	1 (one) <i>hilA</i> <i>Salmonella</i> PCR-positive patient
		<i>Enterobacter sakazakii</i>	1	Negative
		<i>Aeromonas hydrophila/caviae/sobria</i>	1	Negative
		<i>Raoultella terrigena</i>	1	Negative
		<i>Klebsiella oxytoca</i>	1	Negative
		Negative	-	113
Apartadó	Positive	<i>Enterobacter cloacae</i>	3	Negative
		<i>Raoultella terrigena</i>	2	Negative
		<i>Raoultella terrigena</i> , <i>Enterobacter cloacae</i>	1	Negative
		Negative	-	7
Quibdó	Positive	<i>Pseudomonas fluorescens/putida</i>	1	Negative
	Negative	-	1	Negative

PCR: polymerase chain reaction.

(32%), and both *Enterobacter cloacae* and *Raoultella terrigena* were isolated in one (18%) male patient (Table 1).

Pseudomonas fluorescens/putida was the sole isolate from the Quibdó region’s positive patient. *Pseudomonas* spp. (38%), *Klebsiella* spp. (23%), and *Proteus* spp. (9%) were the most frequent species in the Medellín metropolitan area, in addition to unique cases of other bacteria (Table 1).

Salmonella spp. *hilA* PCR

Five (3.3%) of the 149 patients had positive PCR results for the *hilA* gene of *Salmonella* spp., all of whom were female and residents of the Medellín metropolitan area. Two (40%) had positive cultures (*Escherichia coli* and *Pseudomonas fluorescens/putida*) and three (60%) had negative cultures. Table 2 shows the characteristics of *Salmonella* spp. PCR-positive patients.

Discussion

Gallbladder microbiota is a dynamic and complex bacterial community with variability between populations related to geographical, ethnic, and environmental conditions, which additionally could have important clinical implications in the setting of gallstones patients with acute inflammatory complications.

An important finding of our study is the detection of *Salmonella* spp. in 3.3% of the study population, or in all patients from the Medellín metropolitan area. This reveals that the chronic carriage rates in our population are similar to those reported in the literature [15], which further highlights the complexity of *Salmonella* spp. as an infectious agent. There are no a highly efficient, cost-effective, and non-invasive methods for the identification of *Salmonella* spp. chronic carriage. High antibody titer against the Vi antigen is the surrogate

Table 2. Positive *Salmonella hilA* gene PCR, sex, age, and culture results.

<i>hilA</i> PCR	Sex	Age	Culture results
Positive	F	31	Negative
Positive	F	70	Negative
Positive	F	43	<i>Escherichia coli</i>
Positive	F	37	<i>Pseudomonas fluorescens/putida</i>
Positive	F	61	Negative

PCR: polymerase chain reaction.

marker for chronic carriage state, but serology is not a reliable technique [26]. Gallbladder resection and antibiotics can reduce the rates of chronic carriage, but none of these interventions can guarantee a complete cure [27].

It is important to point out the differences between the results of the current and previous studies concerning the frequency of bacterial species, geographical origin of patients, and the Afro-Latin American proportion of the population. In the Medellín metropolitan area, located 1,475 meters above sea level, with an average temperature of 22°C, well-developed public services and sanitation facilities, and a population comprising 19% Afro-Latin Americans [28,29], the most frequent bacteria were *Pseudomonas* spp., *Klebsiella* spp., and *Proteus* spp. In contrast, in Apartadó, located 25 meters above sea level, with an average temperature of 28°C [30], under-developed public services and sanitation facilities, and an Afro-Latin American population of 69% [28,31], the most frequent bacteria were *Enterobacter cloacae* and *Raoultella terrigena*, suggesting differences in the microbiota between populations.

Differences found in our two Colombian populations contrast with the results found in other similar studies from other geographical origins. The relative frequencies of the isolated bacteria in the different studies has shown some consistency within the United States population (*E. coli*, *Enterococcus*, *Klebsiella*, and *Enterobacter*), as was reported by Stewart *et al.* [32,33] and Brook *et al.* [34], and similarities between American and Chilean patients (*E. coli*, *Streptococcus* spp., *Klebsiella* spp., *Enterobacter* spp.) [10].

The results are less homogeneous in the Asiatic surveys from India (*E. coli*, *Klebsiella* spp., *Citrobacter freundii*, and *Salmonella* Typhi) [35] and Sri Lanka (*E. coli*, *Pseudomonas* spp., *Streptococcus* spp., and *Klebsiella* spp.) [36], with important differences when compared to our study. Differences can be explained by the low frequency of *E. coli* and *Enterococcus* and the predominance of species such as *Pseudomonas* and *Klebsiella* found in the studied population.

The variability in the bacterial isolations is a phenomenon related to, in a simplistic manner, differences in the kind of samples used, culture methodologies [37], the anatomical site where the sample was taken, and associated inflammatory conditions [38]. There are variations between the cited studies explained by the previously considered variables. In our study, however, the isolations are different and may be related to the environmental

conditions and the ethnicity of the population factors that produce variations in the microbiome of many anatomical locations [20,21].

As in our study, most of the bacteria isolated in the cited studies belong to the Enterobacteriaceae family and are Gram-negative bacteria, some of which are considered fecal coliforms (*E. coli*, *Klebsiella*, *Enterobacter*, *Raoultella*); these bacteria are ubiquitous in soils, water fonts, vegetables, and contaminated meat products [39]. This kind of bacteria colonize the gastrointestinal tract very early in life, becoming part of the microbiome [40].

High numbers of coliforms in water destined for human consumption correlates to poor water treatment, a condition present among in the Apartadó population and, in much lower proportions, the Medellín metropolitan area. Therefore, to aid in the explanation of the differences among all cited studies and between them and our results, it would be useful to conduct an eco-epidemiological study. This study should use molecular methods to analyze the bacterial communities of the food in a regular diet and the water destined for human consumption in these cities, as well as inside the gastrointestinal tract and gallbladder. The intent would be to establish more objective differences or associations, which would allow for expansion of our knowledge of the microbiome dynamics in our population and the related conditions.

Knowing the core gallbladder microbiota of a population in non-acute inflammatory conditions is an important issue, because some of this bacteria can become pathogenic and there could be no other opportunity to isolate them because microbiota may change, as in the gut, by the inflammatory microenvironment and previous antibiotic use [41].

Acute cholecystitis is a complication of patients with gallstones, and although it is considered a non-life-threatening condition, it may evolve to more serious complaints like empyema, gangrene, and perforation [42]. In cases of acute complications, gallbladder tissue or bile for culture are not available and management options agree on supportive care and early antibiotic use, but delayed cholecystectomy and percutaneous drainage cholecystostomy remain controversial [43-46].

Without results from gallbladder tissue or bile cultures, antibiotic treatment is empiric, relying on the assumption of the probable local microbiota. There have been many studies trying to support the use of antibiotics, with difficulties for comparisons in relation to timing, choice, and duration [47], and disappointing results with respect to benefits (symptoms severity,

perioperative course, post-operative complications, hospital stays) [48,49]. These surveys have not considered the gallbladder microbiota and its variability as a possible source for fluctuations in the proposed outcomes; these fluctuations could be explained by the antibiotic selection and the response for each specific type of microbiota in different populations. Being aware of the probable gallbladder microbiota of the population in the city of medical practice may help optimize the antibiotic selection in acute complications, increasing the benefits of this therapy and even leading to the possibility of avoiding surgery [50].

The low rate of isolations and the lack of anaerobic cultures are weaknesses in our study. Future studies may ameliorate these limitations by including molecular techniques to increase knowledge of the gallbladder microbiota in patients with pathologies such as acute cholecystitis and biliary tract carcinomas in Colombia.

Conclusions

The gallbladder microbiota variability found in the current population belongs to the Enterobacteriaceae family and comprises Gram-negative bacteria, some of which are considered fecal coliforms (*E. coli*, *Klebsiella*, *Enterobacter*, *Raoultella*).

Gallstones, sclerosing cholangitis, and gallbladder carcinoma are pathologies with an unraveled unequal distribution around the world. The gallbladder microbiota and its variability, as in the different Colombian regions studied, could be a key factor in such uneven distribution. A core gallbladder microbiota should be defined, and this definition must be related to geographical, ethnic, and environmental conditions, to support the idea that the microbiota is really associated with gallbladder pathologies. Future studies in other regions of Colombia could exhibit more variability in the microbiota since diet and ethnic origin are quite different. This is the first study of gallbladder microbiota in Colombian patients.

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References

- Shaffer EA (2006) Epidemiology of gallbladder stone disease. *Best Pract Res Clin Gastroenterol* 20: 981-996.
- Stinton LM, Shaffer EA (2012) Epidemiology of gallbladder disease: cholelithiasis and cancer. *Gut Liver* 6: 172-187.
- Stewart L, Grifiss JM, Jarvis GA, Way LW (2006) Biliary bacterial factors determine the path of gallstone formation. *Am J Surg* 192: 598-603.
- Venneman NG, van Erpecum KJ (2010) Pathogenesis of gallstones. *Gastroenterol Clin North Am* 39: 171-183.
- Crawford RW, Rosales-Reyes R, Ramírez-Aguilar Mde L, Chapa-Azuela O, Alpuche-Aranda C, Gunn JS (2010) Gallstones play a significant role in *Salmonella spp.* gallbladder colonization and carriage. *Proc Natl Acad Sci* 107: 4353-4358.
- Toouli J, Bhandari M (2006) Anatomy and physiology of the biliary tree and gallbladder. In Clavien PA, Baillie J, Morse MA, Selzner M, editors. *Diseases of the Gallbladder and Bile Ducts Diagnosis and Treatment, Second Edition*. Massachusetts: Blackwell Publishing. 3-21.
- Rinawi F, Iancu TC, Hartman C, Cohen H, Yarden-Bilavsky H, Lev MRB, Shamir R (2015) Fat malabsorption due to bile acid synthesis defect. *Isr Med Assoc J* 17: 190-192.
- Guyton AC, Hall JE (2006) Secretory Functions of the Alimentary Tract. In Guyton AC, Hall JE, editors. *Textbook of Medical Physiology, Eleventh Edition*. Pennsylvania: Elsevier. 791-806.
- Verdier J, Luedde T, Sellge G (2015) Biliary mucosal barrier and microbiome. *Viszeralmedizin* 31: 156-161.
- Csendes A (1996) Simultaneous bacteriologic assessment of bile from gallbladder and common bile duct in control subjects and patients with gallstones and common duct stones. *Arch Surg* 131: 389-394.
- Liu J, Yan Q, Luo F, Shang D, Wu D, Zhang, Shang X, Kang X, Abdo M, Liu B, Ma Y, Xin Y (2015) Acute cholecystitis associated with infection of Enterobacteriaceae from gut microbiota. *Clin Microbiol Infect* 21: 851.e1-9.
- Peng Y, Yang Y, Liu Y, Nie Y, Xu P, Xia B, Tian F, Sun Q (2015) Cholesterol gallstones and bile host diverse bacterial communities with potential to promote the formation of gallstones. *Microb Pathog* 83-84: 57-63.
- Wu T, Zhang Z, Liu B, Hou D, Liang Y, Zhang J, Shi P (2013) Gut microbiota dysbiosis and bacterial community assembly associated with cholesterol gallstones in large-scale study. *BMC Genomics* 14: 669.
- Karpel E, Madej A, Bułdak Ł, Duława-Bułdak A, Nowakowska-Duława E, Łabuzek K, Haberka M, Stojko M, Okopien B (2011) Bile bacterial flora and its in vitro resistance pattern in patients with acute cholangitis resulting from choledocholithiasis. *Scand J Gastroenterol* 46: 925-930.
- Levine MM, Black RE, Lanata C (1982) Precise estimation of the numbers of chronic carriers of *Salmonella typhi* in Santiago, Chile, an endemic area. *J Infect Dis* 146: 724-726.
- Monack DM, Mueller A, Falkow S (2004) Persistent bacterial infections: the interface of the pathogen and the host immune system. *Nat Rev Microbiol* 2: 747-765.
- Mortimer PP (1999) Mr N the milkster, and Dr Koch's concept of the healthy carrier. *Lancet* 17: 1354-1356.
- Parry CM, Hien TT, Dougan G, White NJ, Farrar JJ (2002) Typhoid fever. *N Engl J Med* 28: 1770-1782.
- Darton TC, Blohmke CJ, Pollard AJ (2014) Typhoid epidemiology, diagnostics and the human challenge model. *Curr Opin Gastroenterol* 30: 7-17.

20. Xu Z, Knight R (2015) Dietary effects on human gut microbiome diversity. *Br J Nutr* 113 Suppl 1: 1-5.
21. Jones ML, Ganopoulos JG, Martoni CJ, Labbé A, Prakash S (2014) Emerging science of the human microbiome. *Gut Microbes* 5: 446-457.
22. Crump JA, Luby SP, Mintz ED (2004) The global burden of typhoid fever. *Bull World Health Organ* 82: 346-353.
23. Cardona-Castro NM, Sánchez-Jiménez MM, Usuga-Silva LY, Arboleda-Naranjo M, Garzón E, Vélez A, Weisner M, Muñoz N, Agudelo CL (2007) Characterization of two typhoid fever outbreaks in Apartadó, Antioquia, 2005. *Bioméd Rev Inst Nac Salud* 27: 236-243.
24. Sánchez-Jiménez MM, Cardona-Castro N (2004) Validation of a PCR for diagnosis of typhoid fever and salmonellosis by amplification of the *hlyA* gene in clinical samples from Colombian patients. *J Med Microbiol* 1: 875-878.
25. Cardona-Castro N, Restrepo-Pineda E, Correa-Ochoa M (2002) Detection of *hlyA* gene sequences in serovars of *Salmonella enterica* subspecies enterica. *Mem Inst Oswaldo Cruz* 97: 1153-1156.
26. Gopinath S, Carden S, Monack D (2012) Shedding light on *Salmonella* carriers. *Trends Microbiol* 20: 320-327.
27. Gonzalez-Escobedo G, Marshall JM, Gunn JS (2011) Chronic and acute infection of the gall bladder by *Salmonella* Typhi: understanding the carrier state. *Nat Rev Microbiol* 9: 9-14.
28. Pastoral Afrocolombiana (2015) Statistics on the Afro-Colombian population - raw. Available: <http://axecali.tripod.com/estadisticas-c.htm>. Accessed 21 October 2015 [Article in Spanish.]
29. Departamento Administrativo Nacional de Estadística DANE Colombia (2005) General census 2005 profile Medellín-Antioquia. Available: <https://www.dane.gov.co/files/censo2005/perfiles/antioquia/m edellin.pdf>. Accessed 22 December 2015.
30. Observatorio Universidad del Valle (2015) Profile municipality of Apartadó. Available : http://prevencionviolencia.univalle.edu.co/observatorios/antio quia/apartado/archivos/perfil_apartado.pdf. Accessed 21 October 2015. [Article in Spanish]
31. Gobernación de Antioquia (2013) The Urabá Antioqueño: a sea of opportunities and potential subregional profile. Available: http://antioquia.gov.co/antioquiav1/organismos/planeacion/de scargas/perfiles/Perfil_Subregional_Uraba.pdf. Assesed 22 December 2015. [Article in Spanish.]
32. Stewart L, Oesterle AL, Erdan I, Griffiss JM, Way LW (2002) Pathogenesis of pigment gallstones in Western societies: the central role of bacteria. *J Gastrointest Surg* 6: 891-903.
33. Stewart L, Griffiss JM, Jarvis GA, Way LW (2007) Bacteria entombed in the center of cholesterol gallstones induce fewer infectious manifestations than bacteria in the matrix of pigment stones. *J Gastrointest Surg* 25: 1298-1308.
34. Brook I (1989) Aerobic and anaerobic microbiology of biliary tract disease. *J Clin Microbiol* 27: 2373-2375.
35. Capoor MR, Nair D, Rajni, Khanna G, Krishna SV, Chintamani MS, Aggarwal P (2008) Microflora of bile aspirates in patients with acute cholecystitis with or without cholelithiasis: a tropical experience. *Braz J Infect Dis* 12: 222-225.
36. Abeyesuriya V, Deen KI, Wijesuriya T, Salgado SS (2008) Microbiology of gallbladder bile in uncomplicated symptomatic cholelithiasis. *Hepatobiliary Pancreat Int* 7: 633-637.
37. Lagier JC, Million M, Hugon P, Armougom F, Raoult D (2012) Human gut microbiota: repertoire and variations. *Front Cell Infect Microbiol* 2: 136.
38. Manolis EN, Filippou DK, Papadopoulos VP, Kaklamanos I, Katostaras T, Christianakis E, Bonatsos G, Tsakris A (2008) The culture site of the gallbladder affects recovery of bacteria in symptomatic cholelithiasis. *J Gastrointest Liver Dis* 17: 179-182.
39. Fleming LR, Bolzan DN, Nascimento JDS (2010) Antimicrobial substances produced by coliform strains active against foodborne pathogens. *Foodborne Pathog Dis* 7: 243-247.
40. Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, Pettersson S (2012) Host-gut microbiota metabolic interactions. *Science* 8: 1262-1267.
41. Lange K, Buerger M, Stallmach A, Bruns T (2016) Effects of antibiotics on gut microbiota. *Dig Dis* 34: 260-268.
42. Portincasa P, Di Ciaula A, de Bari O, Garruti G, Palmieri VO, Wang DH (2016) Management of gallstones and its related complications. *Expert Rev Gastroenterol Hepatol* 10: 93-112.
43. Wu XD, Tian X, Liu MM, Wu L, Zhao S, Zhao L (2015) Meta-analysis comparing early versus delayed laparoscopic cholecystectomy for acute cholecystitis. *Br J Surg* 102: 1302-1313.
44. Koti RS, Davidson CJ, Davidson BR (2015) Surgical management of acute cholecystitis. *Langenbecks Arch Surg* 400: 403-419.
45. Gurusamy KS, Rossi M, Davidson BR (2013) Percutaneous cholecystostomy for high-risk surgical patients with acute calculous cholecystitis. *Cochrane Database Syst Rev* 8: CD007088.
46. Wang CH, Wu CY, Yang JCT, Lien WC, Wang HP, Liu KL, Wu YM, Chen SC (2016) Long-term outcomes of patients with acute cholecystitis after successful percutaneous cholecystostomy treatment and the risk factors for recurrence: a decade experience at a single center. *PLoS One* 11: e0148017.
47. Fuks D, Cossé C, Régimbeau JM (2013) Antibiotic therapy in acute calculous cholecystitis. *J Visc Surg* 150: 3-8.
48. Kanafani ZA, Khalifé N, Kanj SS, Araj GF, Khalifeh M, Sharara AL (2005) Antibiotic use in acute cholecystitis: practice patterns in the absence of evidence-based guidelines. *J Infect* 51: 128-134.
49. Mazeh H, Mizrahi I, Dior U, Simanovsky N, Shapiro M, Freund HR, Eid A (2012) Role of antibiotic therapy in mild acute calculus cholecystitis: a prospective randomized controlled trial. *World J Surg* 36: 1750-1759.
50. Wang CH, Chou HC, Liu KL, Lien WC, Wang HP, Wu YM (2014) Long-term outcome of patients with acute cholecystitis receiving antibiotic treatment: a retrospective cohort study. *World J Surg* 38: 347-354.

Corresponding author

Nora Cardona-Castro (MD, MSc, PhD)
 Department of Microbiology, Faculty of Medicine University CES
 Colombian Institute of Tropical Medicine – University CES
 Calle 10 A No. 22 – 04, Medellín, Colombia
 Phone: (574) 3053500
 Fax: (574) 4035950
 Email: ncardona@ces.edu.co

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