10. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing; 19th informational supplement M100-S19*. Wayne, PA: CLSI, 2009.
11. Pitout JD, Church DL, Gregson DB *et al.* Molecular epidemiology of CTX-M-producing *Escherichia coli* in the Calgary Health Region: emergence of CTX-M-15-producing isolates. *Antimicrob Agents Chemother* 2007; 51: 1281–1286.
12. Pitout JD, Wei Y, Church DL, Gregson DB. Surveillance for plasmid-mediated quinolone resistance determinants in Enterobacteriaceae within the Calgary Health Region, Canada: the emergence of *aac(6′)-Ib-cr*. *J Antimicrob Chemother* 2008; 61: 999–1002.
13. Yamane K, Wachino J, Suzuki S, Arakawa Y. Plasmid-mediated *qepA* gene among *Escherichia coli* clinical isolates from Japan. *Antimicrob Agents Chemother* 2008; 52: 1564–1566.
14. Clermont O, Dhanji H, Upton M *et al.* Rapid detection of the O25b-ST131 clone of *Escherichia coli* encompassing the CTX-M-15-producing strains. *J Antimicrob Chemother* 2009; 64: 274–277.
15. Feil EJ, Li BC, Aanensen DM, Hanage WP, Spratt BG. eBURST:inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. *J Bacteriol* 2004; 186: 1518–1530.
16. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl Environ Microbiol* 2000; 66: 4555–4558.
17. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods* 2005; 63: 219–228.
18. Pitondo-Silva A, Minarini LAR, Camargo ILBC, Darini ALC. Clonal relationships determined by multilocus sequence typing among enteropathogenic *Escherichia coli* isolated in Brazil. *Can J Microbiol* 2009; 55: 672–679.
19. Minarini LAR, Camargo ILBC, Pitondo-Silva A, Darini ALC. Multilocus sequence typing of uropathogenic ESBL-producing *Escherichia coli* isolated in a Brazilian community. *Curr Microbiol* 2007; 55: 524–529.
20. Oteo J, Diestra K, Juan C *et al.* Extended-spectrum beta-lactamase-producing *Escherichia coli* in Spain belong to a large variety of multilocus sequence typing types, including ST10 complex/A, ST23 complex/A and ST131/B2. *Int J Antimicrob Agents* 2009; 34: 173–176.

Phenotypic identification of over 1000 isolates of anaerobic bacteria recovered between 1999 and 2008 in a major Costa Rican hospital

E. Rodríguez-Cavallini, P. Vargas, C. Rodríguez, C. Quesada-Gómez and M.-M. Gamboa-Coronado

Laboratorio de Investigación en Bacteriología Anaerobia (LIBA), Centro de Investigación en Enfermedades Tropicales (CIET), and Facultad de Microbiología, Universidad de Costa Rica, Ciudad Universitaria Rodrigo Facio, San José, Costa Rica

Abstract

Because of limitations in infrastructure, the aetiology of infections caused by anaerobic bacteria is seldom determined in clinical laboratories of developing countries. This study reports on the identification of 1010 anaerobic bacterial isolates collected between 1999 and 2008 in a major Costa Rican hospital with the use of two commercial phenotypic systems (RapID 32A and API 20A). Approximately 60% of the isolates were Gram-positive and, among the 35 species of Gram-positive bacteria found, the genera *Clostridium*, *Propionibacterium* and *Eggerthella*, and anaerobic cocci predominated. Twenty eight species were found among 395 isolates of Gram-negative bacteria. Species of *Bacteroides* were very frequent, followed by species of *Prevotella*, *Veillonella*, *Fusobacterium* and *Porphyromonas*.

Keywords: Anaerobic bacteria, biochemical identification, clinical samples, Costa Rica

Original Submission: 16 September 2010; **Revised Submission:** 13 October 2010; **Accepted:** 28 October 2010
Editor: D. Raoult
Article published online: 3 November 2010
Clin Microbiol Infect 2011; **17:** 1043–1047
10.1111/j.1469-0691.2010.03419.x

Corresponding author: C. Rodríguez, Laboratorio de Investigación en Bacteriología Anaerobia (LIBA), Centro de Investigación en Enfermedades Tropicales (CIET), and Facultad de Microbiología, Universidad de Costa Rica, Ciudad Universitaria Rodrigo Facio, San Pedro de Montes de Oca 2060, San José, Costa Rica
E-mail: cesar.rodriguezsanchez@ucr.ac.cr

Numerous species of anaerobic bacteria proliferate in the surfaces and cavities that make up the human body. This anaerobic microbiota is innocuous under usual conditions; however, it can cause pathology in the head and neck area, the thorax, the abdomen, the skin and soft tissues or the urogenital tract when certain predisposing conditions are present, such as compromised integrity of the skin or mucosae, necrosis resulting from ischaemic processes or the formation of abscesses [1].

The majority of clinical laboratories in Costa Rica and in other developing countries lack the infrastructure and experience required to isolate and identify anaerobic bacteria [2]. Such an absence of knowledge on the aetiology of infections caused by anaerobic bacteria has serious epidemiological repercussions and forces local clinicians to prescribe antibiotic therapy empirically when treating these infections. Moreover, clinicians often follow the trends and recommendations for developed countries, which may not always apply to the epidemiological characteristics of developing societies.

In this note, we retrospectively report on the identity of 1010 anaerobic bacterial isolates from 1061 clinical samples collected in a major Costa Rican hospital between 1999 and 2008. The samples, which were collected through aspiration, drainage or surgical intervention by medical and hospital laboratory personnel, were immediately inoculated in tubes with pre-reduced brain–heart infusion broth containing chopped beef meat [3]. These tubes were incubated at the hospital for 48 h at 35°C before being transported, at room temperature and away from direct sunlight, to the Anaerobic Bacteria Research Laboratory (LIBA) of the University of Costa Rica. Once at LIBA, the tubes were further incubated at 35°C until turbidity was observed, for a maximum of 7 days. Isolation was performed on Columbia agar plates containing 5% blood, 1 mg/L vitamin K and 5 mg/L haemin (BAKH), incubated at 35°C in jars under an anaerobic atmosphere (Anaerogen; Oxoid Hampshire, U.K.). Each of the colonies observed at 48 h was subcultured on three BAKH plates and subjected to an aerotolerance test [3]. The resulting isolates of anaerobic bacteria were identified with the use of a polyphasic algorithm [3] that takes into account their colony and microscopic morphology, reaction to Gram staining, fluorescence under UV light, ability to produce pigments or haemolysis, and RapID 32A or API 20A profiles (bioMérieux Marcy l'Étoile, France).

During the 10 year study period, 1010 isolates were identified from 518 samples positive for anaerobic bacteria (average = 1.9 isolates/sample; Table 1). These results corroborate the polymicrobial nature of infections caused by anaerobic bacteria [4–6]. On the other hand, and in agreement with other studies [4,7], the majority of the isolates were derived from the abdominal cavity, skin, soft tissues or bones (Table 1).

Approximately 60% of the isolates were Gram-positive; of these, 25% exhibited coccoid morphology under the microscope. In total, 35 species of Gram-positive bacteria were identified (Table 2). The majority of Gram-positive bacteria were identified as members of the following genera (in descending frequency): *Clostridium* (*n* = 96; 16%), *Propionibacterium* (*n* = 92; 15%), *Eggerthella* (*n* = 84; 14%) and *Peptoniphilus* (*n* = 76; 12%). The species most frequently found were *Eggerthella lenta* (*n* = 84; 14%), *Propionibacterium acnes* (*n* = 81; 13%) and *Peptoniphilus asaccharolyticus* (*n* = 75; 12%). Most of the 81 strains of *P. acnes* isolated could be skin contaminants. Our results are similar to those obtained by Brook [4] who, in a 12 year study, found 26% of Gram-positive cocci in a group of anaerobic bacteria, with a fair representation of Gram-positive and Gram-negative bacteria. This author and others have reported high frequencies of isolation from clinical samples for the following Gram-positive bacteria: *Propionibacterium* (13–16%), *Clostridium* (7–29%) and *Eggerthella* (20%) [5,7–9]. Most of the Gram-positive bacteria identified were derived from the abdominal cavity, with *E. lenta* (*n* = 44) and species of *Clostridium* (*n* = 42) clearly predominating (Table 2). In this regard, another study dealing with anaerobic infections in a Costa Rican regional hospital showed these two genera of Gram-positive bacteria to be even more prevalent [9]. Many isolates of Gram-positive bacteria were cultivated from skin, soft tissue and bone samples (*n* = 127), from the genitourinary tract (*n* = 113) and from abscess contents (*n* = 109) (Table 2). The genera *Peptoniphilus* (*n* = 23), *Clostridium*, *Propionibacterium* and *Finegoldia* (*n* = 18) predominated in the skin, soft tissue and bone samples. The genitourinary tract samples yielded 24 *Peptoniphilus*, 23 *Propionibacterium* and 21 *Clostridium* isolates, and 14 *E. lenta* isolates were derived from abscess contents (Table 2).

Twenty-eight species were found among the 395 Gram-negative isolates analysed (Table 3). Species of *Bacteroides* predominated overall (*n* = 258; 65%). The other genera found, i.e. *Prevotella* (*n* = 43; 11%), *Veillonella* (*n* = 34; 8%), *Fusobacterium* (*n* = 33; 8%) and *Porphyromonas* (*n* = 27; 7%), were six to eight times less prevalent (Table 3). The majority of the Gram-negative bacteria identified originated from genitourinary samples (*n* = 103), skin, soft tissue and bone samples (*n* = 86), intra-abdominal samples (*n* = 83) or abscess contents (*n* = 81) (Table 3). Bacteria of the genus *Bacteroides* are frequently isolated from clinical samples, with *Bacteroides fragilis* and *Bacteroides thetaiotaomicron* being particularly prevalent [10,11], especially in samples of intra-abdominal origin [12]. The frequency with which this group of bacteria was found in the present study (65%) is greater than that reported at another Costa Rican hospital (40%) [9], at an Estonian hospital (40%) [13] and at a New Zealand hospital (35%) [14], but lower than that reported elsewhere (87%) [4]. As observed in this study, species of *Prevotella* are generally implicated in polymicrobial infectious processes in the urogenital tract [1]. On the other hand, the isolation frequencies of *Veillonella* and *Fusobacterium* reported here are unusually high.

TABLE 1. Isolation source and recovery rate of anaerobic bacteria from clinical samples collected in a Costa Rican hospital between 1999 and 2008

Isolation source	No. of samples positive for anaerobic bacteria	No. of isolates identified	No. of isolates/ sample
Abdominal cavity	147	284	1.9
Genitourinary tract	92	216	2.3
Skin, soft tissues and bones	111	212	1.9
Abscess contents	99	190	1.9
Head and neck	45	70	1.6
Thoracic cavity	22	33	1.5
Blood	2	5	2.5
Total	518	1010	Average = 1.9