Introduction: Anaerobic bacteria represent more than 90% of the human microbiota and are important agents of infections, such as peritonitis and intra-abdominal abscesses, among others. Despite their importance and therapeutic implications, these agents are often not properly detected and identified due to a variety of factors, such as incorrect choice of clinical material, inadequate sample collection and anaerobic techniques. Some authors report an increasing antimicrobial resistance among anaerobes, mainly from the group *Bacteroides fragilis*, and the isolation in culture and correct identification are the indispensable steps for the antimicrobial susceptibility test. Data on the frequency of anaerobes in clinical samples from Brazil are scarce. This work aimed to evaluate the frequency of different anaerobic species isolated from clinical samples in a hospital laboratory in the city of São Paulo.

Method: We performed a retrospective analysis of the laboratory database of all records of cultures for strict anaerobes performed during the period from January 2014 to December 2018. All samples were seeded in thioglycollate broth, on anaerobic blood agar and on selective anaerobic blood agar and were incubated in anaerobic jar at 36°C for up to seven days. Colonies grown on solid media were identified by MALDI ToF MS using the Microflex system (Bruker Daltonics). Results: During the analyzed period, 32,586 cultures were carried out for strict anaerobes. From this total, 992 (3.04%) were positive for strict anaerobes. The most frequently isolated agents were: *Bacteroides fragilis* group 49% (n = 483), *Prevotella* spp. 17.0% (n = 169), *Bacteroides* spp. excluded *B. fragilis* group 14.0% (n = 136), *Finegoldia magna* 5 (N = 10), *Fusobacterium* spp. 3.0% (n = 30), *Cutibacterium acnes* 2.6% (n = 26), *Peptoniphilus* spp. 2.4% (n = 24), *Veillonella* spp. 1.8% (n = 18), *Clostridium* spp.13 1.3% (n = 13) and *Peptostreptococcus* spp.1.0% (n = 10). In conclusion, the most frequently isolated genera were *Bacteroides* (63%) and *Prevotella* (14.0%). Considering that *Finegoldia* and *Fusobacterium*, anaerobes more sensitive to inadequate incubation conditions, had a relevant frequency, it is possible that the low frequency of *Clostridium* is due to sample collection during antimicrobial therapy.

Key words: anaerobes, anaerobic infections, mass spectrometry, fastidious microorganisms.

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