

Bloodstream Infection due to Bacterial Co-Infection in Patients with Rectal Carcinoma: Report of Two Cases

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Abstract

In this study, we reported the bacterial co-infection causing bacteremia in two of 38 patients diagnosed with rectal carcinoma. 10 mL of blood of each patient were cultured in BHI broth. From positive blood cultures, aliquots were streaked on MacConkey agar (aerobiosis), and Bacteroides-bile-esculin agar and kanamycin-blood agar (anaerobiosis). Bacteria were identified by using API-20A kit and confirmed by 16s rDNA sequencing. The bacterial identification showed the presence of *Escherichia coli*, *Bacteroides fragilis* and *Enterococcus faecalis*. The use of DNA sequencing showed to be a tool for rapid screening of blood infections than the classic microbiological methods, and provides an accurate and effective microbial identification in sepsis process. Patients with intestinal malignancy can produce bacteremia with nonspecific or atypical symptoms.

Keywords: Bacteremia; Rectal carcinoma; Bacterial co-infection; Sepsis

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Citation: Castro RLS, Alberca GGF, Freire CA, et al. Bloodstream Infection due to Bacterial Co-Infection in Patients with Rectal Carcinoma: Report of Two Cases. Colorectal Cancer 2016, 2:4.

Received: October 21, 2016; **Accepted:** October 26, 2016; **Published:** November 01, 2016

Introduction

Colorectal carcinoma (CRC) is one of the most commonly diagnosed malignancies and has highly mortality worldwide. This carcinoma has a high incidence in developed countries, and it has increased in Brazilian population, being the second cancer type in women and the third in men [1]. The colorectal cancer affects a segment of large intestine, colon and/or rectum, and most of these tumors begin from polyps growing in the inner layer of the large intestine.

Bacterial sepsis is one of the main causes of morbidity and mortality around the world, and its incidence has increased during the last years in different countries [2]. Aerobic or facultative Gram-positive and Gram-negative bacteria are the most frequent causative agents of bloodstream infection followed by fungal infection [3], and the rapid detection of pathogens in blood of septic patients is essential for adequate treatment and diagnosis.

Currently, blood culture is the standard method for the diagnosis of bacteremia and the final results require at least 48 to 72 hours for aerobic or facultative bacteria. However, anaerobic bacteria require long periods of growing using from 72 hours to 7 days. Moreover, the conventional aerobic culture may cause false-negative results when fastidious or slowly growing organisms are the causative pathogens, such as anaerobes.

Nowadays, molecular methods for rapid identification of pathogens in positive blood culture samples has been used, including conventional and quantitative PCR, DNA microarray, RNA hybridization probes and sequencing [4].

In this study, we reported two cases of bacterial co-infection causing bacteremia in two women with rectal carcinoma.

Case Presentation

Case No. 1

A 70-years-old female patient, smoking, living in São Paulo city, Brazil, was diagnosed with rectal cancer by colonoscopy. At the time of their diagnosis both women presented aqueous diarrhea and bleeding but not fever. Her demographic data showed no

use of illicit drugs, alcohol consumption nor antibiotic therapy, but with familiar history of cancer, mother with stomach cancer and father with lung cancer.

Case No. 2

A 75-years-old female patients, smoking, living in Sao Paulo city, Brazil, was diagnosed with rectal cancer by colonoscopy. At the time of their diagnosis both women presented light diarrhea and no bleeding. This patient showed no use of illicit drugs or antibiotic therapy. Never used alcohol, and her mother developed stomach cancer.

In both cases, patients were attended at the Instituto do Cancer do Estado de Sao Paulo - ICESP (Sao Paulo, SP, Brazil).

In both patients, few minutes before colonoscopy, 10 mL of blood were drawn from cubital vein and immediately inoculated in 100 mL of Brain Heart Infusion (BHI) and incubated in anaerobic conditions (85% N₂, 10% H₂, 5% CO₂) at 37°C for 7 days. After growing period, aliquots of 0.1 mL were immediately transferred to MacConkey agar and incubated in aerobiosis (37°C, 24 h), and Bacteroides-bile-esculin (BBE) agar and kanamycin-blood agar incubated in anaerobiosis (37°C, for 72 h).

On MacConkey agar, lactose-positive colonies of *Escherichia coli* were isolated and identified by conventional PCR using 16S rRNA primers [5]. Media incubated with anaerobic atmosphere showed different types of colonies and they were analyzed by their respiratory type to confirm the presence of anaerobic bacteria. Each characteristic colony obtained from each medium were isolated and identified by using API-20A kit (BioMérieux, Rio de Janeiro, RJ, Brazil) and confirmed by partial sequencing of the 16s DNA gene using standard method with universal primers (Uni-F: 5'-CGC TAG TAA TCG TGG ATC AGA ATG-3' and Uni-R: 5'-TGT GAC GGG CGG TGTGTA-3'). Bacterial DNA from the bacteria was extracted using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions.

Patients did not show gas gangrene, necrosis or soft tissue infection. The bacterial identification showed the presence of *E. coli*, *Bacteroides fragilis* and *Enterococcus faecalis* (Case No. 1), and *Bacteroides fragilis* and *Enterococcus faecalis* (Case No. 2). The results of sequencing indicated closely related species with identity > 99%.

This study was approved by the Ethics Committee of the Medical School of the University of Sao Paulo, SP, Brazil (Process No. 321/14).

Discussion

Hemoculture or blood culture is the current gold standard method for detecting microbial pathogens from blood infections, but this method presents several limitations. Because of their

fastidious characteristic of anaerobic bacteria, their growth takes more time than aerobic or facultative.

Herein, we report a blood co-infection by the three intestinal microorganisms causing bacteremia in two patients with rectal carcinoma. It is of interest since few or no report has been observed in literature. Interestingly, blood sample was obtained before colonoscopy and it is suggested that these microorganisms were able to achieve bloodstream through tumor tissue and they may remain in state of bacteremia for long period [6]. In this case, the infection in rectal cancer involves disruption of the normal barrier due to tumor-induced ulceration, followed by bloodstream invasion, in accordance with Mirza [7] Bacteremia caused by anaerobic bacteria represents from 0.5% to 12% of the total of bacteremia, and the mortality is approximately between 25% and 44% [8]. Studies have shown that bacterial community in an individual is relatively stable along the distal digestive tract [9]. However, bacterial composition changes in rectal mucosal associated with colorectal adenomas may have an influence in the adenoma formation. In addition, with the mucosal disruption bacteria can be spread hematogenously.

Historically, *E. faecalis* was responsible for the majority of clinical enterococcal infections [10] however, Gudiol [11] reported the high prevalence of *Enterococcus faecium* and a low frequently of *E. faecalis* in blood infection from patients with cancer. In both reported cases, *E. faecium* was not detected.

In many cases conventional blood cultures are negative in the face of strong clinical indicators of sepsis and further examinations are required. If laboratories do not receive the indications of a possible suspect of anaerobic infections, it can lead to lack of positive results.

The presence of *E. coli*, *B. fragilis*, and *E. faecalis* in blood suggests that these resident bacteria of the intestinal microbiota were able to achieve the bloodstream through tumor lesion. Interestingly, the patients did not show apparently discomfort, such as fever or body pain. Since malignant tumors can occur as long as several years after infection its evaluation is imperative, in accordance with Wentling [12] the use of DNA sequencing showed to be an effective tool for rapid screening of blood infections than the classic microbiological methods, giving an accurate, rapid and effective microbial identification in sepsis process for choosing an adequate therapy. In conclusion, patients displaying intestinal malignancy can produce bacteremia with atypical symptoms.

Acknowledgements

This study was supported by Grant from FAPESP 2013/13652-6. The authors thank Mrs. Marcia Harumi Fukugaiti for her technical support.

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