braz j infect dis. 2015;19(6):571–577

The Brazilian Journal of
INFECTIOUS DISEASES

www.elsevier.com/locate/bjid

Original article

Molecular characterization of microbial contaminants isolated from Umbilical Cord Blood Units for transplant

Juan Manuel Bello-López[a,*], *Jorge Noguerón-Silva*[a], *Jorge Ismael Castañeda-Sánchez*[b], *Julieta Rojo-Medina*[a]

[a] *Centro Nacional de la Transfusión Sanguínea, México D.F., México*
[b] *Universidad Autónoma Metropolitana, unidad Xochimilco, México D.F., México*

ARTICLE INFO

Article history:
Received 28 May 2015
Accepted 9 July 2015
Available online 9 September 2015

Keywords:
Umbilical Cord Blood Units
Microbial contamination
Molecular characterization

ABSTRACT

Disposal of Umbilical Cord Blood Units due to microbial contamination is a major problem in Cord Blood Banks worldwide as it reduces the number of units available for transplantation. Additionally, economic losses are generated as result of resources and infrastructure used to obtain such units. Umbilical Cord Blood Units that showed initial microbial contamination were subject to strains isolation, identification, and characterization by sequencing the 16S rRNA gene and Enterobacterial Repetitive Intergenic Consensus (ERIC-PCR). Moreover, tests of antimicrobial resistance/sensitivity and phenotypic activities that may play an important role in microbial infection were performed. Microbial contamination was detected in 120 Umbilical Cord Blood Units (2.31%) in the period from 2003 to 2013. The most frequently isolated strains were *Enterococcus faecium*, followed by *Staphylococcus epidermidis*, *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus haemoliticus*, *Klebsiella pneumoniae*, *Enterococcus durans*, *Lactobacillus helveticus*, *Enterococcus hiriae* and *Roseomonas* genomospecies 5. The ERIC-PCR assays revealed a wide genetic diversity in some strains although belonging to the same genus and specie, indicating different sources of contamination. Broad-spectrum penicillins, third generation cephalosporins, aminoglycosides, and fluoroquinolones showed lower inhibitory activity on the tested strains. All strains were proteolytic, 67.69% were amylase-positive, 27.6% hemolysis-positive, and 34.71% nuclease-positive. The most common sources of contamination were: vaginal flora, digestive tract, and skin flora, highlighting the need for staff training in good manufacturing practices in collection SCU since all contaminants identified are part of the microbial flora of the donors. Implications and consequences in the therapeutic use of Umbilical Cord Blood Units for transplantation contaminated by multiresistant bacteria in immunocompromised patients are discussed.

© 2015 Elsevier Editora Ltda. All rights reserved.

* *Corresponding author at*: Centro Nacional de la Transfusión Sanguínea, Av. Othón de Mendizábal 195, Col. Zacatenco, C.P. 07360 México, D.F., Mexico.
E-mail address: juanmanuelbello81@hotmail.com (J.M. Bello-López).

http://dx.doi.org/10.1016/j.bjid.2015.07.005
1413-8670/© 2015 Elsevier Editora Ltda. All rights reserved.

Introduction

In order to ensure Umbilical Cord Blood Units (UCBU) supply and safety, suitable for transplantation, it is necessary to have strict quality controls. These controls include: CD34+ cell count, typing by Human Leukocyte Antigen (HLA), serological tests, clonogenic capacity, and microbiological monitoring.[1–3] Collection, manipulation, cryopreservation, and transplantation of UCBU involve a large number of procedures that are carried out in different areas and can result in microbial contamination of the final UCBU. For the most part, the collection of Cord Blood (CB) is carried out in an operating room with the use of safety procedures such as skin disinfection, closed collection systems, and sterile equipment among others. However, microbial contamination can occur during this process. Besides manipulation, cryopreservation, and thawing provide chances for the introduction of bacteria into UCBU.[4] Additionally, several sources of contamination have been described, e.g. vaginal flora, skin, poor aseptic techniques in the areas, use of contaminated material, and others.[5,6] It has been reported that the rate of contamination of UCBU ranges from 0 to 48%.[7,8] The use of UCBU in sterile conditions is of vital importance because patients requiring this type of transplants are severely immunocompromised. Therefore, these patients have a high risk of contracting infections associated with the transplant. Previous studies have shown that the use of contaminated UCBU is one of the causes of morbidity and mortality in these patients.[9] The identification of sources of contamination is crucial, from the sampling of CB until its processing in the laboratory. In previous research, identification of the contaminants was performed by phenotypic characteristics, such as biochemical tests, serotypes, and antimicrobial susceptibility.[10,11] More recently, molecular typing methods, such as Enterobacterial Repetitive Intergenic Consensus (ERIC), based on Polymerase Chain Reaction (PCR) "ERIC-PCR",[12,13,14] have replaced previous techniques allowing better epidemiological determination of contamination sources. This molecular method is fast, simple, and highly reproducible. It also has been widely used in a large number of microbial types as well as in the study of clonality and identification of contamination sources. In addition, the new molecular technique for detecting bacteria allows for a quick and easy identification, compared with the classical microbiological methods. In this study we implemented the use of 16s rRNA gene sequencing and ERIC-PCR for typing the microbial strains obtained from criopreservated UCBU in the CB bank (CBB) of the National Center of Blood Transfusion (NCBT) in a period of 11 years (2003–2013) to identify potential sources of microbial contamination and carry out measures for their prevention. Tests of antimicrobial resistance/susceptibility and phenotypic activities that may play an important role in microbial infection were performed for the isolated strains. Implications and consequences of the therapeutic use of UCBU for transplantation contaminated by multiresistant bacteria in immunocompromised patients are discussed.

Materials and methods

A retrospective analysis over 11 years of cryopreserved UCBU in the CBB of the CNTS was performed. A total of 120 UCBU known to be contaminated by bacteria (aerobic, anaerobic, or both) were identified and extracted from the tank of liquid nitrogen (Bioarchive™ System TG 3626). They were immediately immersed in water bath at 37 °C for thawing.

Enrichment of microbial contaminants in the BacT/ALERT 3D automated system

All sampling was carried out under aseptic conditions in a laminar flow cabinet. All content (25 mL) of each UCBU thawed was obtained by puncture with a hypodermic syringe. 12.5 mL of the UCBU content were directly inoculated into the BacT/ALERT 3D: FA (FAN aerobic) and FN (anaerobic FAN) bottles (bioMérieux, Nueringen, Germany). Negative controls were established by inoculating 2 mL of UCBU previously tested negative into BacT/ALERT 3D bottles. Over the seven-day incubation period, samples that exhibited a positive signal of contamination by the unit monitor of the BacT/ALERT 3D system were included in the study.

Isolation of microbial strains

Bottles that showed positive signals of contamination were subcultered in solid media: 5% sheep blood agar, chocolate agar, Eosin Methylene Blue agar (EMB), mannitol salt agar, *Pseudomonas* agar, Trypticase Soy Agar (TSA), and Sabouraud Dextrose Agar (SDA). The plates were incubated aerobically at 37 °C for 24–48 h and at 28 °C for 24–72 h (only for SDA plates). The gas-pack system (BD GasPakTM EZ Gas Generating System) was used in the production of an anaerobic environment for the isolation of anaerobic bacteria in blood agar and in chocolate agar at 37 °C for 48–72 h. Subsequently, microbial strains were purified in the LB agar. All strains were cultured in LB-broth, then frozen in glycerol (50%) and, stored at −70 °C. For molecular biology assays, total DNA from all strains was extracted as described using the QIAamp DNA Mini QIAcube Kit (QIAGEN, Germany).

Genetic identification

All the amplification reactions were performed in a Touchgene Gradient thermal cycler Gene Amp® PCR System 9700 (Applied Biosystems). Polymerase chain reactions of the 16s rRNA gene were performed with universal primers 27F (5′-AGA GTT TGA TCM TGG CTC AG-3′) and 1492R (5′-TAC GGY TAC CTT GTT ACG ACT T-3′) using the conditions recommended by DeSantis et al.[15] Amplicons were analyzed on horizontals 1% agarose gels using 1× Tris–Borate–EDTA buffer (TBE), purified and sequenced by the Biology Institute, Universidad Nacional Autónoma de México (UNAM) using an ABI PRISM® 310 Genetic Analyzer sequencer (Applied Biosystems, California USA). Nucleotide sequences were compared with

Download English Version:

https://daneshyari.com/en/article/3343869

Download Persian Version:

https://daneshyari.com/article/3343869

Daneshyari.com