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SHORT COMMUNICATION

Enterococcus hirae: a zoonotic microorganism in human umbilical cord blood

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Abstract *Enterococcus hirae* is rarely collected from man, while it is a common pathogen in mammals and birds. We describe the first isolation of the organism (strain DSM 27815) from human umbilical cord blood (UCB), thus emphasizing the risk of contamination of UCB units for clinical use. In this context, we also highlight the importance of an extensive training of the collecting personnel as to the observance of the disinfection protocol ensuring UCB units sterility.

Introduction

During the last years many banking facilities have been established worldwide; over 600,000 cord blood units (CBUs) have been stored and more than 30,000 umbilical cord blood (UCB) transplantations performed, accounting for approximately 20 % of allogeneic haematopoietic stem cell transplants (HSCTs) (Ballen et al. 2013). Compared to other haematopoietic sources of stem cells, cord blood (CB) shows particular logistic and clinical advantages such as lack of donor risks, faster availability, higher frequency of ethnic minorities, greater tolerance of 1–2 HLA mismatches out of 6, lower incidence and severity of Graft versus Host Disease (GvHD), and reduced risk of infectious agents transmission. On the other hand, there are disadvantages such as low content of haematopoietic stem cells in the CBUs, risk of HSCT failure, or delayed engraftment, greater risk of infectious complications, unavailability of the donor for a second donation in case of graft failure, or for lymphocyte collection if disease relapses. Again, at the time of donation or during the follow-up, eventual donor haematologic/immunologic disorders may be unknown. Many efforts have been made to overcome these problems, including better selection criteria for a suitable CBU, use of specific conditioning regimens, transplantation of double CBUs, intrabone infusion, ex vivo cell expansion and accreditation of the CB banks to guarantee compliance with the NetCord FACT International Standards. Therefore, UCB transplantation is constantly encouraged.

International standards and regulations require that microbial screening be carried out on all CBUs banked for clinical use and that contaminated products be discarded. Nevertheless, literature reports a significant risk of microbial contamination (0–48 %) (Clark et al. 2012) and, despite accurate disinfection protocols, this still is a cause of concern as final product minimum volume to be tested has not yet been defined; furthermore, the volume sampled is generally lower than that recommended by the manufacturers, with consequent risk of inadequate detection sensitivity.

The genus *Enterococcus* comprises 37 different species, among which *Enterococcus faecalis* and *Enterococcus faecium* are characterized, in medical microbiology, by the highest frequency of occurrence as well as their well

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known pathogenic potential (Chan et al. 2012). Conversely, limited attention has been given to species other than *E. faecalis*/*E. faecium*, as they show a relatively low prevalence and are difficult to identify (Tan et al. 2010). Enterococci are an essential part of the enteric microflora both in human and animals; also, they can be found in vagina and the oral cavity (Chan et al. 2012) as harmless commensals and are employed in the preparation of alimentary products, such as cheese and sausages (Foulquié Moreno et al. 2006). Nonetheless, the importance of infections they cause has been gaining the interest of epidemiologists and microbiologists and, moreover, the generation of drug-resistant strains of the genus has been increasingly described as a threaten (Chan et al. 2012; Savini et al. 2012). Enterococcal disease comprises upper and lower airway, wound, hepatobiliary, intra-abdominal and urinary tract infections (UTIs), meningitis, infective endocarditis and bacteraemia (including neonatal sepsis). Particularly, enterococci are important agents of nosocomial UTIs and, when endocarditis is excluded, the urinary tract has to be considered as the potential source of an enterococcal bloodstream infection (Chan et al. 2012; Savini et al. 2012; Tan et al. 2010).

Enterococcus hirae incidence among human enterococci is 1–3 %, that is one of the lowest for the genus (Chan et al. 2012). The species was first described by Farrow and Collins in 1985, when it was observed to be an agent of growth depression in young chickens, and is nowadays known to cause disease in several animals and birds (Chan et al. 2012; Talarmin et al. 2011). A very limited number of cases has been documented in humans, instead, although misidentification may have occurred, leading to underestimation (Chan et al. 2012). Particularly, *E. hirae* has been reported to cause wound infections and gastritis, as well as a few episodes of bacteraemia (sources for some of which were infected native and prosthetic heart valves, a spondylodiscitis, urinary and biliary tract infections) (Chan et al. 2012; Talarmin et al. 2011; Tan et al. 2010). Instead, isolation from blood products for transfusion or transplantation has never been reported, and we describe here the first observation of the organism from human UCB.

Materials and methods

A UCB unit was collected at the Pescara Cord Blood Bank (PeCBB) collection site after a vaginal delivery with placenta in situ by gravity in a closed bag system (Macopharma-MSC-1202PU, France); a trained midwife carried out the collection after cord antisepsis with 70 % isopropanol (PVS, Italy) and 10 % povidone iodine (Esoform, Italy). Informed consent was previously obtained from the parents as per an ethical committee-approved protocol. According to national policy, only units with an adequate cell number are banked for clinical use, that is, currently, those with an initial content of 15×10^8 nucleated cells and a final content of 12×10^8 nucleated cells, after removing most of plasma and red blood cells.

Current PeCBB policy for banked UCB units recommends the inoculum of 2 ml of final product into a standard anaerobic culture bottle for adults and 2 ml into a paediatric culture bottle for aerobes; for further safety, a 5 ml-sample of plasma and 5 ml of red blood cells obtained by product processing are inoculated into anaerobic and aerobic adult bottles. In addition, to control the collection process and, particularly, the maintenance of competence of collecting personnel, the PeCBB performs a sterility check on non-banked units; therefore, one unit per operator per month undergoes sterility control.

Accordingly, due to the low cell content, the PeCBB discarded the mentioned unit from clinical use, and employed it for the operator monitoring. Hence, a 10 ml-sample was inoculated into two BacT/Alert (bioMérieux, France) adult bottles, one for anaerobes and one for aerobes. After 24 h incubation, the instrument detected both as positive.

Aliquots from the positive bottles underwent Gram staining as well as inoculation onto sheep blood agar plates (Liofilchem®, Italy), overnight, at 36 °C, aerobically. The isolate identification was achieved through Lancefield antigen detection (StreptoSlide, Diesse Diagnostica Senese s.p.a., including group A, B, C, D, F and G antisera), and the Vitek2 GP card (bioMérieux), at the bacteriology laboratory of the Spirito Santo Hospital of Pescara, Italy; then, it was sent to the Department of Biomedical Sciences, Campus Biomedico, University of Rome (Italy), where characterization through the API20 Strep (bioMérieux), the Phoenix GP (BD, Becton–Dickinson, US), and 16S rDNA-sequencing was performed. Finally, antibiotic susceptibilities were determined (by agar disc test) according to 2012 European Committee for Antibiotic Susceptibility Testing (EUCAST) guidelines.

Results

As explained above, a UCB unit was discarded from clinical use, owing to the low cell content, and destinated to the operator monitoring by the handling CB Bank. Hence, 10 ml were inoculated into two BacT/Alert adult bottles (one for anaerobes and one for aerobes), to check the unit sterility, and were both detected to be positive after 24 h incubation.

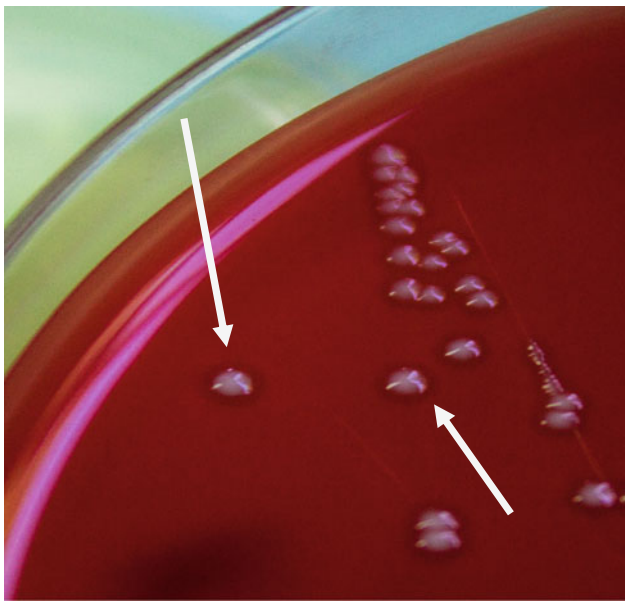

Fig. 1 *Eh61* culture on a sheep blood plate: thin, α-haemolytic bands (i.e. those indicated by *arrows*) are visible around colonies

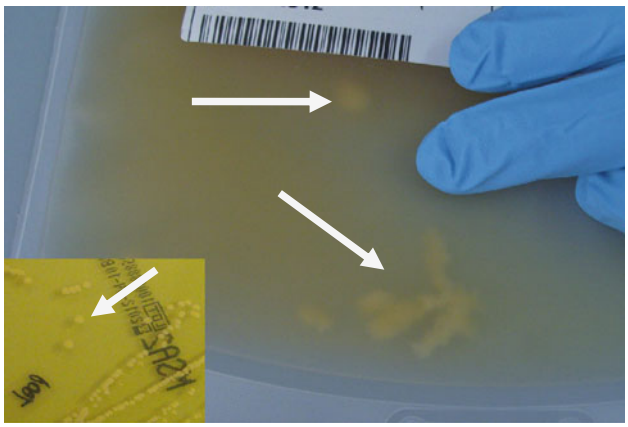

Fig. 2 *Main image* Platelet unit (from Dept. Transfusion Medicine, Spirito Santo Hospital, Pescara, Italy) contaminated by *Staphylococcus aureus* (macroscopically visible bacterial clusters are indicated by *arrows*)—picture taken by Daniela Astolfi (coauthor). *Small image S. aureus* colonies on mannitol salt agar cultivated from the above mentioned platelet unit (the *arrow* indicates a colony)

Gram positive cocci were observed in each of the samples, while cultures yielded catalase negative, PYR-positive colonies showing a thin, α-haemolytic band (Fig. 1) as well as the Lancefiled group D antigen. Identification as *E. hirae* (99 % certainty) was provided both by the GP card and the Phoenix GP, while 16S rDNA-sequencing showed 100 % sequence homology with *E. hirae* strain JQ735956. Conversely, classification as *Enterococcus durans* (89 %) was achieved by the API20 Strep (identification with such a method is considered to be reliable if obtained with ≥90 % certainty). The strain showed susceptibility to ampicillin, glycopeptides (vancomycin, teicoplanin) and linezolid. It was deposited into the laboratory strain collection as *Eh61*, and to DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH), Braunschweig, Germany, under accession number DSM 27815. Finally, according to the PeCBB policy, the midwife who collected the contaminated unit received a written notification concerning the positive sterility test and was recommended to carefully follow the disinfection protocol before future UCB collection procedures.

Discussion

Bacterial contamination may involve any blood component for transfusion and transplantation, although it is mostly observed with platelets (Fig. 2). Since, as a consequence, recipient's device colonization and/or bacteraemia, even fatal, may occur after transfusion/transplant of contaminated products, blood components must be examined carefully to ensure their sterility prior to clinical use (Guo et al. 2011; Savini et al. 2010).

According to current literature, the overall incidence of UCB contamination is 0–48 %. It is mainly due to skin and gut flora (38.4 and 33.9 %, respectively), while environmental contaminants or vaginal saprophyte bacteria are involved to a lesser extent (6.5 and 1.8 %, respectively). A higher rate is finally observed after vaginal delivery (5.4 %) rather than post-caesarian section (1.1 %) (Clark et al. 2012).

A further variable that is associated with a more frequent reporting of UCB contamination is the use of bottles for anaerobes in addition to those designed for organisms growing aerobically, while inoculating paediatric bottles (that only provide aerobic atmosphere and allow to introduce a smaller volume of the final product than those for adults) is associated with a reduced sensitivity when contaminating organisms are anaerobes (Clark et al. 2012). Again, Clark addressed the role of quality improvement strategies, as well as of training and expertise of personnel as a tool to reduce the contamination risk (Clark et al. 2012).

As *E. hirae* is a zoonotic pathogen, we presumed the donor acquired colonization from pets. Accordingly, the mother referred past exposure to cats, birds and turtles. Hence, it has been assumed that the organism had colonized her enteric flora thus contaminating the CB unit at the time of delivery, during collection, probably due to a non-thorough disinfection procedure. Nevertheless, the donor was not asked to collect stools for culture, after contamination detection, aiming not to create unjustified alarmism and anxiety. Moreover, transient colonization

due to ingestion of *E. hirae*-contaminated fruit and/or vegetables could not be excluded.

This brief communication wants to emphasize the observation of a veterinary organism in the checked product, along with the risk of UCB contamination during delivery. *E. hirae* may be hard to identify through automated, phenotype-based systems (bioMérieux Rapid ID 32 Strep, for instance) that potentially misinterpret it as *E. faecium*, *E. durans* or *Enterococcus gallinarum* (Talarmin et al. 2011). We obtained unreliable results through API20, but correct identification was provided by both the Vitek2 GP card and the Phoenix. Nevertheless, it is clear that molecular tools are indispensable to confirm identification of uncommonly encountered enterococci.

Potential transmission of enterococci to blood component recipients is of worrisome concern, as these pathogens poorly respond to several antibiotic classes and can therefore represent difficult-to-treat agents of disease once 2012). *E. hirae* is mostly an animal pathogen and isolation from a UCB unit raises questions about its source(s). Accordingly, we believe that asking informations about contact with animals and country environments might be included in the donors' anamnesis once zoonotic microorganisms are isolated, to investigate origins of UCB contamination by veterinary bacteria, and elucidate their life cycle, niches, and routes of transmission.

Conflict of interest None.

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