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CASE REPORT

Lactococcus lactis blood products contamination resulting in fatal human case: insights from a forensic case

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SUMMARY

Lactococcus species are micro-aerophilic Gram positive bacteria characterized by low virulence features and other biotechnological properties of industrial interest. They are thus widely employed in food fermentation processes. Despite its low pathogenic potential and food grade safety, *L. lactis* may, however, rarely cause infections, especially among immunocompromised hosts. Moreover, the growing complexity of patients implies increased detections of such infections. This said, there is a paucity of data concerning *L. lactis* infections from infusion of blood transfusion products.

To our knowledge, this is the first case of *L. lactis* infection from transfusion of blood products, as observed in an 82-year-old Caucasian male undergoing weekly platelet and blood transfusion due to sustained severe thrombocytopenia. Albeit minimally pathogenic, *L. lactis* should be considered for thorough testing, especially in the case of human-derived infusion products such as platelets due to their storage requirements for extended times at room temperature and their use in immunocompromised and critically ill subjects.

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INTRODUCTION

Lactococcus species are micro-aerophilic Gram positive cocci and rod-shaped bacteria known together as lactic acid bacteria (LAB). Their low virulence, coupled with their ability to produce lactic acid and lower pH along with other biotechnological properties of industrial interest, have allowed their use in a considerable number of food manufacturing processes (Aisnworth *et al*., 2014; Mercier Bonin *et al*., 2017), with safety profiles emerging from long-term use in the food industry (Bron *et al*., 2018). Specifically, *Lactococcus lactis* is widely employed as starter culture in food fermentation processes (Mercier Bonin *et al*., 2017), and interest in its use as a probiotic along with drug delivery is also rising (Mercier Bonin *et al*., 2017, Bron *et al*., 2018). Moreover, *L. lactis* does not naturally inhabit human gut. However, it has demonstrated an ability to adhere to cell surface mucins and transiently colonize the gastrointestinal tract (Mercier Bonin *et al*., 2017).

While different Lactococcus species exist, early evidences dating back to the '90s report infections ascribable to a variety of Lactococci. Once known as *Streptococcus lactis*, *Lactococcus lactis* was subsequently reclassified in the *Lactococcus* genus in 1985 (Schleifer *et al*., 1985) Indeed, the first reported case of *L. lactis* infection dates back to when the pathogen was still referred to as Streptococcus lactis (Mannion *et al*., 1990).

Despite its low pathogenic potential and food grade safety, *L. lactis* may be causative of infections, especially in the immunocompromised host. As a result, a growing body of evidence is currently available concerning *L. lactis* sustained infections, possibly due to the increasing use of organic unpasteurized products as well as the growing complexity of patients (Hadjisimeou *et al*., 2013; Shimizu *et al*., 2019), whereby difficulty in testing may concur to a possible under-diagnosis (Shimizu *et al*., J 2019).

That said, there are currently no publicly available

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reports of *L. lactis* infections from infusion of blood transfusion products. The aim of this article is to report the evidence of a case of infection and sepsis due to *L. lactis* from transfusion of blood products, with a revision of similar cases from the literature.

CLINICAL CASE

An 82-year-old Caucasian male undergoing weekly platelet and blood transfusion due to sustained severe thrombocytopenia was admitted to the Emergency Department (ED) due to onset of fever, hypotension and vomiting after being transfused with one unit of platelets and one unit of red blood cells. The patient was started on infusions of both platelets and red blood cells and immediately developed signs and symptoms of septic shock. He was therefore urgently managed for suspicion of septic shock.

The patient's medical history was remarkable for myelodysplastic syndrome, liver cirrhosis due to chronic hepatitis C virus (HCV) infection with sustained virological response after treatment with sofosbuvir and daclatasvir, type 2 diabetes mellitus, long-standing persistent atrial fibrillation and Barrett's esophagus.

Upon ED admission, the patient presented with chills and confusion. Body temperature was 40.0°C, blood pressure was 80/40 mmHg, and the pulse rate was 110 BPM. Oxygen saturation at ambient air was 100%. Physical examination of chest, heart and abdomen was unremarkable. Clinical biochemistry and hematology assessments performed upon ED admission were as follows: white blood cell count of 5700/µL (differential count: 95.5% neutrophils, 4.2% lymphocytes), normocytic and normochromic anemia (hemoglobin of 8.1 g/dL), platelet count of 12000 cells/µL, C reactive protein (CRP) 0.34 mg/dl (reference range <0.50), procalcitonin 40.0 ng/ml, prothrombin time percentage activity 35% (reference range 80-130%), international normalized ratio (INR) 2.34, activated partial thromboplastin time ratio (aPTT) 2.32 (reference range 0.81-1.2); alanine aminotransferase (57 units/L), aspartate aminotransferase (55 units/L) and creatinine (1.62 mg/dL) were mildly elevated, electrolytes were within normal ranges. The patient underwent imaging evaluation. Chest plain radiograph was normal. CT pulmonary angiography (CTPA) showed filling defects within the segmental and subsegmental pulmonary vasculature of the right lower lobe and no airspace opacifications. Three sets of blood cultures were drawn and the patient was started on intravenous piperacillin/tazobactam therapy (4.5 g/t.i.d.). The patient was subsequently admitted to the Intensive Care Unit due to recognition of sepsis in order to undergo aggressive fluid resuscitation. Due to the onset of septic shock, the patient was started on vasopressors and underwent orotracheal intubation and mechanical ventilation; antimicrobial therapy was escalated to prolonged infusion of meropenem, vancomycin continuous infusion and caspofungin. Throughout the following three days, clinical conditions progressively deteriorated, and the patient expired due to sepsis-related multiple organ failure.

The two blood cultures yielded positivity to *L. lactis*, whereby susceptibility results reported as minimum inhibitory concentrations (MICs) were as follows: penicillin =0.5 mg/L, ampicillin =0.5 mg/L, ceftriaxone =0.5 mg/L, meropenem =0.12 mg/L, vancomycin =0.5 mg/L. Positive blood cultures therefore prompted analysis of the transfused blood products, which had been retrieved immediately after being discarded due to the patient's reaction.

Indeed, immediately following transfusion, both the platelet and the red blood cell bags were thrown into the medical waste bin (traces of previous patient's vomit were also present). Aerobic enrichment culture of material aseptically drawn from one line of the red blood cell bag grew *L. lactis*. The same bacterium was recovered from aerobic and anaerobic enrichment cultures of material collected from the platelet bag (the bag was pierced after accurate disinfection with chlorhexidine). The time to positivity (TTP) of the culture of red blood cells was 19.26 hours, while the TTP of the culture of platelet material was 6.19 hours. Fractionated plasma material from the same donor did not yield the same results and was negative for *L. lactis*. The donor was a regular blood donor and exempt from infection and symptoms thereof at the time of blood apheresis.

Whole genome sequencing analysis performed confirmed 100% clonal identity of all 3 strains recovered. After death, the corpse was autopsied to confirm cause of death.

Macroscopically, the autopsy showed cerebral edema and congestion, bilateral pleural effusion and minimal pericardial effusion. The posterior part of the right ventricle showed red spots. At the pulmonary level, there was congestion and imposing edema associated with red spots in the lower lobes of the lungs.

Histological analysis revealed massive cortical edema, diffuse vasogenic edema, acute vascular congestion, cerebellar edema, disruption of Purkinje's cells, as well as pulmonary edema, cardiac edema and diffuse glomerulosclerosis associated with acute tubular necrosis.

DISCUSSION

The autoptic observations commonly attributed to sepsis are generally non-specific and present local signs of inflammation or ischemia, such as myocarditis, pulmonary edema and infiltration, cerebral swelling and tubular necrosis in the kidney.

Also in this case, the autopsy was thus able to confirm all the elements required for the diagnosis of septic shock. However, as signs were non-specific, a multi-

Table 1 - *Published cases of bacteremia due to L. lactis reported in the literature.*

Author	*Species*	*Age (years)*	*Diagnosis*	*Treatment*	*Outcome*	*Country*
Durand *et al.*	*L. lactis*	69	Bacteremia	Cefotaxime + Amikacin	Recovered	France
Karaaslan *et al.*	*L. lactis*	0	Bacteremia	Vancomycin + Cefepime → Vancomycin	Recovered	Turkey
Karaaslan *et al.*	*L. lactis*	0	Bacteremia	Vancomycin	Recovered	Turkey
Gurley *et al.*	*L. lactis*	59	Bacteremia	Ertapenem → Amoxicillin	Recovered	USA

disciplinary approach was required in order to identify the causative agent.

L. lactis infections in the immunocompetent host are scarce though increasingly recognized, and there are currently no reports of *L. lactis* sepsis from fractionated plasma products. Only one recently published experience reporting infections due to *L. garvieae*-contaminated donor platelet concentrates (PC) is available in the literature (Colagrossi *et al.*, 2022). The authors describe 3 cases of sepsis in pediatric patients following PC infusion from one blood donor. The PC were infused in 4 onco-hematological and immunocompromised pediatric patients, 3 of whom developed sepsis due to the same strain of *L. garvieae* within 24 h from transfusion, with the exception of the first patient, possibly due to the lag time for bacterial growth following thawing of the product.

In our case, *L. lactis* was detected only in the cellular products (fractionated plasma was negative). Despite the lack of virulence factors normally present, other cases of *L. lactis* blood stream infections and sepsis have been described and are available in the literature (Karaaslan *et al.*, 2015; Karaaslan *et al.*, 2016; Shimizu *et al.*, 2019). For a recent case review, refer to Shimizu *et al. Table 1* depicts all published cases of bacteremia due to *L. lactis* reported to date in the literature. As with other organisms, *L. lactis* can acquire plasmids, and has been reported to acquire different subsets of plasmids conferring adhesive and resistance properties along with potential virulence factors and resistance determinants (Ainsworth *et al.*, 2014; Mercier Bonin *et al.*, 2017). As a gastro-intestinal (GI) tract colonizer, it may potentially also acquire other determinants from common GI colonizers such as *E. faecium* (Palmer *et al.*, 2020).

Hence, it is possible that *L. lactis* originally translocated asymptomatically from the donor to the bloodstream, subsequently adhering to the surface of red blood cells and platelets, as it can withstand gastrointestinal conditions and stay viable following ingestion (Hadjisymeou *et al.*, 2013). *L. lactis* can survive low temperatures, and some strains can survive freeze drying processes. Moreover, survival is increased when cells are placed at 4°C (Sanders *et al.*, 1999). This could explain the rapid growth upon infusion of both PC and red blood cells. Despite therapeutic intervention, the bacterial load was possibly too elevated in order to reach adequate dosing and timely treatment. Platelet concentrates have been reported to harbor the highest risk of infection, sepsis, and death compared to other blood transfusion products, whereby bacterial contamination and septic transfusion reactions account for the majority of attributable morbidity and mortality associated with their use. Skin-derived contamination along with their storage modality implying the use of gas-permeable bags kept at room temperature (20-24 C) with continuous agitation facilitate bacterial growth, as opposed to other plasma fractionated products requiring freezing (Levy *et al.*, 2018).

The Italian National surveillance system provides annual epidemiological data concerning adverse events and infections of hemoderivatives and fractionated blood products, whereby only viral infections such as HIV, HCV and other viral infections are reported, thus possibly under-reporting potential other infective causes (Catalano *et al.*, 2020). Moreover, repeated blood infusions and iron overload exposes such subjects to further susceptibility. HCV and comorbid patients are notably immunocompromised subjects prone to infections. ROS species production due to iron overload are common traits of myelodysplastic syndromes (Medvedev *et al.*, 2016; Petzer *et al.*, 2021), whereby iron overload also increased susceptibility to infections and contributing to organ dysfunction (Zou *et al.*, 2017; Toma *et al.*, 2012). Indeed, *L. lactis* strains have previously been reported to produce Reactive Oxygen Species (ROS), in particular extracellular $O_2^{\cdot-}$, possibly conferring growth advantage in the GI tract and favoring colonization (Huycke *et al.*, 1996). Platelets have also been implied in pathogenesis and infection, and their role in generating a pro-oxidative and pro-coagulant environment during infections is well-established (Assinger *et al.*, 2014). It is therefore not surprising they may have played a fundamental role in this case. Furthermore, we postulated that the platelet bag was the source of infection, according to the short TTP in platelet material culture, and that the red blood cells bag was contaminated following transfusion. The fact that platelets are stored in agitation at room temperature might confirm this hypothesis.

CONCLUSION

The multidisciplinary approach, through clinical, microbiological, and autoptic data analysis, confirmed the septic shock diagnosis.

This is the first case report of *L. lactis* sepsis from PC and red blood cell transfusion in an adult subject. De-

spite being considered safe and non-pathogenic, *L. lactis* infections, albeit rare, are being increasingly reported, and should hence be considered for thorough testing, especially in an era of technological improvement. This experience calls for increased awareness of testing requirements on human-derived infusion products, as potential pathogens may escape attentive testing and management. This is especially true for PC, due to their storage requirements for a prolonged time at room temperature, and their potential to cause severe infections in critically ill and immunocompromised subjects.

Knowledge of the pathogenetic mechanism underlying the reaction is essential not only for defining appropriate prevention strategies, but also for medico-legal purposes. Indeed, there are appropriate technologies to sterilize platelet bags by UV irradiation, using a technology similar to photopheresis. However, these technologies are expensive and therefore not always accessible to all territorial structures, but only to a few national reference centers.

On the other hand, since the events described above, thanks to changes in the Italian Society of Transfusion Medicine (SIMTI) guidelines, the four/six-monthly microbiological culture controls on skin, instruments, storage fridges and platelet shakers have been introduced (Albolino *et al.*, 2019). Culture quality checks on expiring bags are also performed at regular intervals to understand if additional contamination has occurred.

Authors' contribution

AS, SG review design and manuscript preparation; LM, ACM, literature review and manuscript preparation; FS: editing and manuscript preparation; CT, MDP: review design, literature review and manuscript preparation. All authors read and approved the final version of the manuscript.

Disclosures

CT has received funds for speaking at symposia organized on behalf of Pfizer, Novartis, Merck Gilead, Zambon, Infectopharm, Sionogy, Menarini, Angelini and Astellas. All other authors: None.

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