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TRANSFUSION

CASE REPORT

Collaboration is key: Case report of suspected *Pseudomonas fluorescens* transfusion-associated infection

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Abstract

Background: We report a case of suspected *Pseudomonas fluorescens* transfusion-transmitted infection in a 64-year-old female patient with pancreatic adenocarcinoma. The patient developed a biliary obstruction necessitating a drainage catheter, which was complicated by an arterial hemorrhage. Following the transfusion of 1 RBC unit, the patient developed flank pain, chills, and tachycardia.

Study Design and Methods: The transfusion reaction workup was negative for hemolytic transfusion reaction. However, Gram stains of the implicated RBC unit revealed Gram-negative rods. Fortunately, the patient was already receiving broad-spectrum antibiotics, and preliminary investigation results were available early enough to alert the medical team and adjust antibiotic coverage. The patient was hospitalized in the ICU where she had elevated WBC counts that normalized after the addition of cefepime. The blood collection center was notified.

Results: Both aerobic and anaerobic cultures incubated at 35°C were negative for growth. A subculture and incubation at room temperature (25°C) demonstrated sufficient growth for the identification of *Pseudomonas fluorescens* by both Vitek-MS MALDI-TOF and Vitek2 biochemical methods.

Discussion: *Pseudomonas fluorescens* is a Gram-negative rod-shaped bacterium, well-studied as an environmental microbe. It can cause opportunistic infections in humans and was implicated in previous fatal septic transfusion reactions. This report highlights the importance of both standardization in blood product culture protocols and the need for collaboration between microbiology laboratories and transfusion practitioners to optimize the recovery of potentially clinically important fastidious organisms.

KEYWORDS

infectious disease testing, RBC transfusion, transfusion-transmitted disease – bacteria

1 | CASE PRESENTATION

We report herein the case of a 64-year-old female with metastatic pancreatic adenocarcinoma who developed biliary obstruction and underwent an endoscopic retrograde cholangiopancreatography (ERCP) with a left hepatic duct stent placement and a right-sided internal-external biliary drainage catheter. During her hospitalization, her hemoglobin (Hb) level dropped from 9 to 5.9 g/dL. A CT scan revealed possible arterial hemorrhage for which she was re-operated. During surgery, the massive transfusion protocol (MTP) was activated, and the patient received several units of RBC, platelets, and cryoprecipitates. The surgery was uncomplicated, with a successful biliary tube exchange, and the patient was transferred to the ICU.

During the transfusion of the last RBC unit of the MTP, the patient developed flank pain, chills, and tachycardia (increase in heart rate from 93 bpm to 173 bpm), along with a rise in blood pressure from 123/61 mmHg to 142/88 mmHg, but no fever (temperature remained between 37° and 37.5°C). Blood transfusion was stopped immediately; a total of 181 mL was transfused over a period of 75 min. A transfusion reaction workup was initiated.

1.1 | Transfusion reaction workup/investigation

No clerical errors were found. The RBC bag passed the visual inspection, with no signs of hemolysis. The expiry date on the RBC bag was checked, and the unit was labeled to expire 18 days after the transfusion date. Antibody screen and direct antiglobulin test (DAT) were negative, and crossmatching was compatible for pre- and post-transfusion samples. The post-transfusion urine analysis sample was negative for RBCs, leukocytes, and bacteria. There was no evidence of a hemolytic transfusion reaction, and the patient's Hb increased from 6.3 to 8.1 g/dL post-transfusion.

Gram stains and culture of the returned component bag were performed per the institution's standard operating procedure for investigating transfusion reactions. As per our institutional protocol, the blood bag sampling process consists of unit port disinfection and sampling of 3–4 mL of blood for Gram stain and blood bottle inoculation. Gram stain demonstrated a few Gram-negative bacilli. The medical team was notified immediately to closely monitor the patient. Blood cultures were taken from the patient before cefepime was administered. Concurrently, the blood collection center was notified immediately after the Gram stain results were obtained, followed by the culture findings. The investigation performed by the blood collection center revealed that only one donor/one product was implicated in this transfusion. No significant findings were reported upon record review, and no donor follow-up was performed.

The patient was stable in the ICU and receiving treatment for other complications, including suspected pulmonary embolism and pulmonary infection. Since she was already receiving vancomycin (15 mg/kg Q12H IV) and metronidazole (500 mg Q 8H IV) for other infectious conditions, the medical team decided to add cefepime (2 g Q 8H IV) 48 h after the Gram stain results. Notably, two sets of blood cultures were taken from the patient before cefepime was added. The patient was managed appropriately with an infectious disease team consultation. Her WBC count was trending up from $22.4 \times 10^{\wedge 3}/\mu L$ at baseline to $79.8 \times 10^{\wedge 3}/\mu L$ with a left shift of 89.5% neutrophils after the transfusion, but it started to decrease 24 h after cefepime administration to $61.7 \times 10^{\wedge 3}/\mu L$ (Day 2 post reaction) and $55.7 \times 10^{\wedge 3}/\mu L$ (Day 3 post-transfusion), eventually stabilizing around $23.1 \times 10^{\wedge 3}/\mu L$ 3 days after the transfusion. Standard blood cultures were followed daily, but both aerobic and anaerobic final cultures of the implicated RBC unit, and the patient's blood showed no growth at 5 days.

Due to high clinical suspicion of a septic transfusion reaction, additional investigation was carried out. The initial blood culture bottle was re-Gram stained and again revealed Gram-negative bacilli, ruling out sample contamination in the initial slide preparation.

Despite these findings, the culture of the implicated RBC unit on trypticase soy agar with 5% sheep blood remained negative at routine incubation temperatures (35°C). No special culture media was used to recover *Pseudomonas fluorescens*; however, subculture and incubation at room temperature (25°C) demonstrated growth sufficient for identification. Using both Vitek-MS MALDI-TOF and Vitek2 biochemical methods, the isolate was identified as *P. fluorescens* with a probability of correct identification of 99 to 99.9%. Antibiotic susceptibility testing (AST) was performed on Muller-Hinton agar per institutional and Clinical Laboratory Standards Institute (CLSI) disc diffusion/E-Test protocol both at 35°C and 25°C. *P. fluorescens* growth was only demonstrated at the latter. As the test method deviated from CLSI standardized incubation temperature, minimum inhibitory concentrations were reported without interpretation.

2 | DISCUSSION

Despite a robust hemovigilance program in the United States to ensure blood safety,

transfusion-transmitted infections (TTI) are still occurring, resulting in morbidity and mortality in blood product recipients. From 2016 to 2020, 13% of FDA-reported fatalities were due to microbial contamination.[1,2] In another report, bacterial infections accounted for around 69% of TTI, mainly in platelet components, with a predominance of *Staphylococcus* species and viridans-group *Streptococcus* species.[3] Unlike platelets that require routine bacterial risk mitigation through bacterial testing and/or pathogen reduction, RBC units have no additional bacterial risk mitigation in place in the US, other than donor history screening and checking vital signs. Moreover, bacterial contamination could be under-reported as the extent to which febrile transfusion reactions are investigated differs from one institution to another, often meeting difficulty in assigning imputability due to the absence of patient's blood culture or discordance with other testing or the clinical picture.[4] The Hemovigilance Module Surveillance Protocol V2.8 from the National Healthcare Safety Network (NHSN) defines a definitive TTI as a "Laboratory evidence of a pathogen in the transfusion recipient"[5] which can be questionable in some cases where an organism was recovered in blood product and not the recipient, or when the clinical picture/symptomatology strongly hints at the presence of a certain pathogen that was not cultured or didn't grow in the microbiology laboratory. In this report, we are reporting a suspected TTI from an RBC unit contaminated with *P. fluorescens*.

P. fluorescens is a Gram-negative, rod-shaped bacterium, belonging to *the Pseudomonas* genus, closely related to *P. putida* and *P. aeruginosa*[6] and is well-studied as an environmental microbe. *P. fluorescens* has been implicated in transfusion-transmitted infection and transfusion-associated fatality. Based on a US report in the early 80s, three separate septic transfusions occurred from RBC units contaminated with *P. fluorescens*. Two of them resulted in fatality.[7] *P. fluorescens* was also involved in a septic transfusion reaction in 1984 in France[8] and Australia.[9] In 1986, two transfusion-related septicemia events occurred in the United Kingdom following transfusion of RBC units contaminated with *P. fluorescens*.[10] Another fatal transfusion reaction with *a P. fluorescens*-contaminated whole blood-derived platelet unit was also reported in the United Kingdom in 1988.[11] From 2013 to 2021, the FDA reported 3 fatalities caused by RBC contaminated with *P. fluorescens*, among the total of 7 fatalities associated with RBC contaminated with bacteria during this time. Of note, all except one reported *P. fluorescens* septic transfusion reaction was associated with RBC units. *P. fluorescens* has been isolated as a cold-growing Gram-negative organism from the skin of multiple blood donors.[12]

Additionally, *P. fluorescens* can cause opportunistic infections in humans, with reported cases of indwelling catheter infections through biofilm formation,[13] pseudobacteremia,[14] cystic fibrosis respiratory infections,[15] and a possible role in inflammatory bowel diseases.[16] *P. fluorescens* has been reported to be responsible for a multistate outbreak of bloodstream infections after exposure to contaminated heparinized saline flushes prepared by a compounding pharmacy, further lending credence to its role as a common environmental contaminant.[13]

P. fluorescens is an obligatory aerobe, with a permissive growth range from 4°C to 32°C for most non-mammalian samples and at higher temperatures, up to 37°C for human isolates.[17] *P. fluorescens* isolates can be either psychrotrophic, with a maximum growth temperature above 20°C, or psychrophilic, with a maximum temperature for growth of 20°C or lower.[17,18] As reported by Khabbaz et al., the three isolates that were implicated in the septic transfusion reaction had optimal growth at 25°C to 30°C and the slowest growth at 37°C.[7] In line with this report, the *P. fluorescens* isolate from the implicated RBC unit in our case did not grow at 35°C but did grow at 25°C. It is more likely psychrophilic, but we cannot determine that definitively since no other temperatures were tested.

In this case report, the patient's clinical presentation was suspicious of a septic transfusion reaction, from the initial presentation of chills and tachycardia to significant elevation of WBC, with left shift, post transfusion. Fortunately, the patient was receiving broad-spectrum antibiotics, and the transfusion reaction workup, including the Gram stain and culture of the implicated unit was fast enough to notify the medical team and adjust antibiotic coverage quickly, and patient recovered, including the return of WBC counts to baseline. However, we cannot determine with certainty that this is a septic transfusion reaction, since the patient's blood cultures post transfusion were negative. The latter can be explained by the following: (1) The patient was already on antibiotics at the time of the transfusion, (2) The patient's blood culture was only performed with standard 35°C incubation, which was more likely a non-optimal culture condition for this case. Additionally, the reaction may be caused by endotoxins; unfortunately, no measurement of endotoxins was performed. Lack of co-components from the same donation provides no additional aid to the investigation. Due to the possible psychrophilic and common environmental contaminant nature, the origin of the *P. fluorescens* isolate involved in this case is more likely environmental or a skin contaminant, unlikely to be silent bacteremia in the donor. Environmental contaminates have been implicated in septic transfusion

reactions, such as the reported *Acinetobacter* contamination in Platelet Collection Set Manufacturing Facility.[19]

Case imputability and the source of contamination were difficult to assess with certainty due to the following limitations: (1) Gram stains of the blood component bag were not quantified; (2) the patient's blood culture was not performed at 25°C like the blood component, and the patient was on antibiotics prior to blood culture sampling; (3) no endotoxin was measured for the blood component bag; (4) no co-component to provide additional supportive evidence; (5) the possibility of sample contamination cannot be ruled out completely; (6) the isolate was not saved to perform an environmental epidemiology study, such as nucleic acid sequencing.

Since 1980, microbiology laboratories have identified such rare pathogens either by "trial and error", or by "hit or miss" and whenever *P. fluorescens* is identified, susceptibility patterns differ greatly at different incubation temperatures,[10] leaving microbiologists hesitant about providing results to clinicians and further delaying optimal patient's care.

To date, the importance of this report is not in the novelty of the organism isolated, but in highlighting the role of the microbiology laboratory in the detection of these pathogens in transfused blood products, which is often faced with multiple challenges, including low residual volumes and a high rate of contamination following the administration of blood products.[20] The American Society of Microbiology guidelines recommend incubating blood samples at 35°C and at lower temperatures for samples from cellular therapy products,[21] but some pathogens escape the normal growth pattern, resulting in a false-negative culture. Since no single set of incubation temperatures would capture all possible agents of transfusion-mediated bacterial sepsis, we recommend the need for extensive collaboration between specialists in transfusion medicine and microbiology, both to standardize specific protocols for transfusion/transplant unit incubation and, failing that, to generate transparent culture protocols in which both transfusion medicine and microbiology have a shared stake. Additionally, the following measures may be considered to aid the investigation and determine imputability: (1) Perform semi-quantitation Gram stains and/or CFU of culture; (2) modify or optimize culture conditions for both the blood component and the patient's blood culture, including a range of indicated temperatures, when clinical suspicion does not match laboratory findings; (3) measure the level of endotoxin in the blood component bag; (4) perform similar investigations on co-components if available; (5) rule out the possibility of sample contamination; (6) save all isolates for possible environmental epidemiology study, such as nucleic acid sequencing.

3 | CONCLUSION

Whenever the clinical presentation does not correlate with any laboratory result, further investigations are necessary. Bacteria in general, and *Pseudomonas* species specifically, are well known for their wide growth temperature range. Such rare but potentially life-threatening pathogens should not be missed in clinical laboratory investigations. In addition, blood collection centers may need to implement more stringent quality measures, as contamination can occur at any stage of blood manufacturing, distribution, and storage. Lower incubation temperatures for red cell product cultures implicated patient or donor may need to be considered when clinical suspicion is high for TTI. It is also paramount to audit periodically the bedside blood product administration practices of clinical staff, starting from the unit handoff at the blood bank to the spiking of the unit by the nurse, with an emphasis on education to avoid or minimize the risk of contaminating organisms.

CONFLICT OF INTEREST STATEMENT

We declare no conflicts of interest.

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