Contamination of Bone Marrow Products with an Actinomycete Resembling Microbacterium Species and Reinfusion into Autologous Stem Cell and Bone Marrow Transplant Recipients

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Bacterial contamination of bone marrow or peripheral blood stem cell transplant products typically occurs with skin flora or, rarely, gram-negative organisms. We describe a clonal outbreak of contamination in transplant products caused by contamination with an aerobic actinomycete that occurred at our institution during the summer of 2001. From 1 July through 12 September 2001, 73 peripheral blood or bone marrow stem cell products were obtained from 39 patients, and 34 products were found to be contaminated with the outbreak strain. Fourteen patients were reinfused with contaminated cells, and the outbreak strain was isolated from the blood cultures for one patient. Investigation revealed multiple potential sources for contamination during the product cryopreservation process. The outbreak of contamination was aborted upon modification of the cryopreservation process.

The combination of high-dose chemotherapy and autologous peripheral blood stem cell or bone marrow transplantation has become a standard treatment option for many hematologic malignancies [1]. The obtention, processing, and storage of transplant product involves several steps typically performed in different locations by different staff. Manipulation during processing, either before or after cryopreservation, may potentially result in bacterial contamination. Bacterial contamination of transplant product has been previously described, and it affects <1%–45% of transplant products [2–7]. Depending on the contaminating organism, reinfusion of contaminated product can have serious medical consequences.

We describe an outbreak of contamination with an actinomycete closely resembling Microbacterium and Plantibacter species secondary to contaminated autologous transplant product that occurred at our institution in the summer of 2001. The actinomycetes are aerobic, non–spore forming, irregularly shaped, gram-positive rods usually isolated from environmental sources [8]. Traditionally, these organisms have not been considered human pathogens; however, some Microbacterium species have rarely been reported to cause disease in patients with cancer [9, 10].

The Princess Margaret Hospital (Toronto, Ontario, Canada) specializes in the treatment of solid and hematologic malignancies. In 2001, the Autologous Stem Cell Transplant Program performed transplantations for 176 patients with hematologic malignancies, including multiple myeloma (58% of patients), non-Hodgkin lymphoma (23% of patients), Hodgkin lymphoma (10% of patients), and acute myeloid leukemia (4.5% of patients). In August 2001, the transplantation
unit informed the Infection Prevention and Control Unit (IPAC) of 4 autologous transplant recipients whose cultures of transplant product obtained at the time of reinfusion had grown gram-positive bacilli. In all cases, the products had been re-infused into patients before it was known that they were contaminated. The isolates were initially identified as *Corynebacterium* or *Aureobacterium* species using the API Coryne test strip (bioMérieux) and were considered to be skin flora contaminants; however, this identification was later revised, as described below in Results.

Follow-up blood cultures for the 4 initial patients showed no bacterial growth. However, on 6 September 2001, cultures of transplant product that was obtained from a fifth patient after reinfusion grew a “coryneform” bacterium, which was later determined to be identical to the first 4 isolates. Given these findings, we undertook an outbreak investigation to determine the source of the contamination.

**MATERIALS AND METHODS**

The outbreak investigation consisted of 5 simultaneous components: identification of the outbreak organism using fatty acid methyl ester (FAME) analysis and 16S rDNA sequencing, investigation of the reinfusion process, investigation of the cryopreservation process, a retrospective microbiological survey of cryopreserved transplant products, and molecular fingerprinting of isolates by PFGE.

**Organism identification.** After their initial isolation and subculturings on 5% sheep blood agar (Oxoid) to ensure purity, all coryneform isolates noted on Gram stain were preliminarily identified by assessing atmospheric and temperature growth requirements, catalase production, bile esculin hydrolysis [11], and the API Coryne strip (bioMérieux). Further identification was performed by FAME analysis using Sherlock, version 4.0 (Microbial ID) [12], and 16S rDNA sequencing, in accordance with the method of Schmidt and Relman [13], at the Health Canada National Microbiology Laboratory (Winnipeg, Manitoba) and the IPAC research laboratory at the University Health Network (Toronto, Ontario).

**Investigation of the reinfusion process.** Infection-control staff observed several transplant product reinfusion procedures to determine compliance with recommended infection-control practice. Staff were interviewed, and samples of their hand flora were obtained for culture. Environmental samples were obtained for culture from all equipment used during the reinfusion process by means of sterile culture swabs premoistened with sterile water. Environmental samples were plated on colistin naladixic agar (Oxoid) and then inoculated into sodium thioglycolate broth and incubated in room air at 30°C for 24 h. The broth culture was then subcultured on 5% sheep blood agar (Oxoid).

**Investigation of the cryopreservation process.** On several occasions, infection-control staff and a stem cell transplant technologist familiar with the cryopreservation process visited the outside laboratory where the cryopreservation procedure was performed. The cryopreservation protocol was documented and laboratory technique was observed during the processing of several specimens. Staff were interviewed with respect to their roles, equipment cleaning, recent protocol changes, equipment changes, and recent building maintenance or construction. Samples were obtained for culture from the hands of staff, all equipment used during the cryopreservation process, media and consumable supplies, and other possible environmental sources, including ventilation ducts and potted plants.

**Microbiological survey of cryopreserved transfusion product.** To determine the extent of the outbreak of contamination, cryopreserved transplant product processed during the period of December 2000 through July 2001 was cultured, as described above, for the presence of the outbreak strain.

**Molecular fingerprinting.** The relatedness of the isolates was determined by PFGE. Agarose plugs were prepared and then digested with *Xba*I restriction endonuclease. Electrophoresis was performed on a CHEF-DR II apparatus (Bio-Rad) using the following parameters: 1% agarose gel; temperature, 12°C; switch time, 5–35 s; run time, 20 h; voltage, 200 V. Epidemiologic-related isolates were compared with control strains of *Curtobacterium* and *Corynebacterium* species obtained from the American Type Culture Collection. The criteria reported by Tenover et al. [14] were used to determine the relatedness between isolates.

**RESULTS**

**Identification of the outbreak organism.** The outbreak strain demonstrated small, yellow-pigmented colonies after 48 h of incubation at 30°C. Growth was partially inhibited by incubating the strain at 37°C in 5% CO₂. Gram staining revealed small, thin, pleomorphic, gram-positive rods. The organism was strictly aerobic and catalase and bile esculin positive. The index outbreak strain was initially identified as a member of the genus *Corynebacterium* or the genus *Aureobacterium* using the API Coryne test strip (bioMérieux), with a biotype number of 0452004. This biotype number was consistent at all of the investigating laboratories.

The organisms most closely related to the outbreak strain on FAME analysis are shown in table 1. Although the outbreak organism most closely resembled *Curtobacterium* species, the results were not conclusive, and 16S rDNA typing was performed for 4 isolates (table 2). Although the outbreak strain most closely matched *Microbacterium* species, it was also highly related to other actinomycetes.

**Product reinfusion.** Transplant products are kept frozen...
Table 1. Organisms that were most closely related to the outbreak strain, as determined by fatty acid methyl ester analysis.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Similarity indexa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curtobacterium species</td>
<td>0.391, 0.394, 0.438</td>
</tr>
<tr>
<td>Bacillus circulans</td>
<td>0.301, 0.332, 0.363</td>
</tr>
<tr>
<td>Aureobacterium species, Corynebacterium aquaticum, and Clavibacter xylic</td>
<td>0.281, 0.319, 0.322</td>
</tr>
</tbody>
</table>

NOTE. Each sample was tested in triplicate, and each row shows the 3 readings.

a A similarity index of 1.00 is considered to be a perfect match. A similarity index of >0.3 with a separation of 1.0 between the first and second choice is considered to be a good library comparison. A similarity index of 0.3–0.5 and well separated from the second choice may be a good match but an atypical strain. A similarity index of <0.5 suggests that the species is not in the database.

b All 3 organisms have the same similarity indices.

until the time of reinfusion, when they are thawed at the bedside in a water bath (temperature, 40°C) in a sterile plastic bag (figure 1). The water bath is filled with fresh tap water immediately before infusion, and it is cleaned with an accelerated hydrogen peroxide–based cleanser/disinfectant (Virox; Virox Technologies) between each procedure. At the completion of the procedure, the final rinse is sent to the microbiology laboratory for culture (culture point “C”).

Although it was initially hypothesized that contamination may have occurred during the reinfusion process, observation failed to reveal any significant breaks in infection-control technique, and cultures of environmental samples of equipment, staff hands, the water bath, and liquid nitrogen tanks failed to yield the outbreak strain.

During the initial investigation, one patient’s preprocessing cultures grew coagulase-negative staphylococci (culture point “A”). In accordance with the transplantation protocol, a tube of frozen transplant product (culture point “B”) was submitted for culture to determine whether the product itself was contaminated or whether the positive culture result merely represented contamination of the culture with skin flora. The frozen transplant product cultures yielded the outbreak strain, suggesting that contamination occurred during the cryopreservation process.

Cryopreservation process. After they are obtained, mononuclear cells are separated and delivered to an outside laboratory for processing and freezing (figure 1). The plasma is removed from the product after centrifugation, and a sample is sent for culture (culture point “A”). A freezing mixture is then freshly prepared, consisting of 40% autologous plasma, 40% Media 199 (Gibco), and 20% dimethylsulphoxide (Edward Scientific), and the mixture is added in equal volume to the stem cells.

Autologous bone marrow is obtained from patients for whom peripheral stem cell collection was not successful. Bone marrow is collected into heparin-containing transfer packs and immediately transported to the outside laboratory for additional processing. It is filtered under a laminar flow hood through a sterile fine needle and prepared for cryopreservation.

The buffy coat is obtained using a centrifugal cell separator, and cultures are sent to the microbiology laboratory (culture point “A”). The same freezing mixture as above is added in equal volume to the bone marrow, which is then frozen. For both procedures, ~2 mL of the cell-freezing mixture preparation is frozen separately from the main cryopreserved product should additional testing (i.e., cytogenetics) be required (culture point “B”).

Investigation of the cryopreservation process revealed several potential sources of contamination. In early July, the Media 199 used in the cryopreservation process was changed from a single-use preparation to a multiple-use bottle. Laminar flow hoods were cleaned only at the end of the day and not before and after manipulation of the product. Isopropyl alcohol was used to wipe the work surface once per day before commencement of work for the day. Finally, the pipetting technique used to obtain media for blood product processing was less than ideal. Specifically, media were obtained using a sterile syringe rather than sterile pipettes, and multiple entries into the bottle were sometimes performed using the same syringe. No equipment changes had been performed recently, and there was no history of recent renovation or building maintenance in the area.

Cultures of staff hand flora and environmental specimens obtained from 2 laminar flow hoods, the centrifuge, ventilation ducts, potted plants in the laboratory, and all other equipment used in the processing of the products failed to yield the outbreak strain. However, one culture of a sample of the laminar flow hood grew an organism resembling Cellulomonas species, and another specimen yielded Corynebacterium species. These organisms were frozen for later PFGE analysis. Opened bottles of Media 199 from the same lot number were culture negative.

Table 2. Organisms that are most closely related to the outbreak strain, as determined by 16S rRNA typing.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Matching score</th>
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<tbody>
<tr>
<td>Microbacterium species LB030</td>
<td>1358</td>
</tr>
<tr>
<td>Plantibacter flavus</td>
<td>1354</td>
</tr>
<tr>
<td>Uncultured bacterium Q2-28C8</td>
<td>1330</td>
</tr>
<tr>
<td>Microbacterium species</td>
<td>1259</td>
</tr>
<tr>
<td>Unnamed Curtobacterium species</td>
<td>1259</td>
</tr>
<tr>
<td>Aureobacterium testaceur</td>
<td>1259</td>
</tr>
<tr>
<td>Curtobacterium species</td>
<td>1251</td>
</tr>
<tr>
<td>Glacial ice bacterium</td>
<td>1249</td>
</tr>
<tr>
<td>Microbacterium foliorum</td>
<td>1243</td>
</tr>
<tr>
<td>Unnamed Microbacterium species</td>
<td>1243</td>
</tr>
</tbody>
</table>
Figure 1. Flow chart showing transplant product collection, processing, and reinfusion procedures. Media 199 is manufactured by Gibco. DMSO, dimethylsulphoxide (Edwards Scientific).

as were 2 unopened bottles, although no opened bottles remained that would have been used in the preparation of contaminated product.

**Microbiological survey of cryopreserved transfusion product.** To determine the extent of the outbreak, the decision was made to culture samples of frozen product obtained from all patients who underwent procedures starting on 1 December 2000 (table 3). Thirty-five products of peripheral blood stem cells and bone marrow were found to be contaminated with the outbreak strain from the period of 1 July 2001 through 12 September 2001. This period coincided with the switch from single-dose to multiple-dose media bottles.

**Molecular fingerprinting.** Twenty-two isolates of the outbreak strain and 2 environmental isolates were assessed by PFGE (figure 2). All outbreak isolates were identical by XbaI restriction digest. The environmental isolates, Corynebacterium species, and an organism resembling Cellulomonas species had different PFGE profiles.

**Follow-up for the outbreak investigation.** On 12 September 2001, IPAC made several recommendations to the outside laboratory responsible for processing the transplant product. It was recommended that the Media 199 be filter sterilized into single-dose aliquots to avoid contamination of the multiple-use bottles. Laboratory staff were educated as to proper sterile technique to reduce the risk of media contamination. It was also recommended that the contaminated laminar flow hood not be used until a maintenance evaluation was conducted. The evaluation revealed that the laminar flow hood high-efficiency particulate air filter was blocked, resulting in decreased airflow. The laminar flow hood was repaired, and a cleaning protocol was developed. Specifically, the hood was to be cleaned with an accelerated hydrogen peroxide cleanser/disinfector (Virox) before starting work, as well as before and after the processing of any products. It was also suggested that all plants be removed from the laboratory. Finally, it was recommended to change the routine culture protocol such that cultures would be sent to the laboratory after product manipulation rather than before, so that contamination during processing could be detected before the product was reinfused.

With the implementation of these recommendations, the outbreak of contamination stopped immediately. No additional contamination of transplant product with the outbreak strain or other bacteria has occurred during the ensuing 9 months.

In total, 14 patients were infused with contaminated trans-
plant product during the outbreak. In 5 patients, the contaminated product was unknowingly infused, whereas, in another 9 patients, re-collection of the bone marrow or peripheral blood stem cells was not a clinical option. The decision was made to reinfuse contaminated product with prophylactic vancomycin administration. Of the 5 patients who were reinfused without receipt of prophylactic vancomycin, only the fifth patient developed apparently transient bacteremia. The patient developed a fever within hours of completing the reinfusion procedure, and cultures of blood samples obtained during the febrile episode also yielded the outbreak organism. The fever resolved within hours of starting therapy with vancomycin and piperacillin/tazobactam. Although the patient subsequently had a prolonged course of febrile neutropenia and probable pulmonary aspergillosis, the outbreak strain was not isolated from a clinical specimen after the first positive blood culture result.

**DISCUSSION**

The potential for bacterial contamination of peripheral blood stem cells or bone marrow is well known. Smith et al. [2] described a 36% contamination rate for autologous bone marrow before processing, which increased to 43% after thawing. Farrington et al. [3] reported a bone marrow contamination rate of 39.5% and a peripheral stem cell contamination rate of 18% before cryopreservation. In both studies, skin flora, particularly coagulase-negative staphylococci, were the most commonly isolated organisms. Lazarus et al. [5] reported lower rates of contamination, and, unlike the findings from other health care centers, gram-negative organisms were predominately isolated. Prince et al. [4] experienced a 2.2% rate of culture positivity in their autologous bone specimens, but no organisms were isolated from peripheral blood stem cells. Again, skin flora was the predominating organism. In all of these studies, few episodes of bacteremia resulted from contaminated product being reinfused into patients, which suggests that the cultures, rather than the products, may have been contaminated, or that the products were contaminated with low levels of organisms.

The manufacture of sterile blood products requires appropriate standards and techniques to be used to prevent microbial contamination. There are enormous potential consequences for immunocompromised patients should they receive contaminated transplant product. Peripheral blood stem cells and bone marrow are difficult to process in a closed system because of the need to add cryopreservation reagents yet maintain sterile conditions [15]. The Foundation for the Accreditation of Hematopoietic Cell Therapy [16] and the US Food and Drug Administration [17] provide collection, processing, and quality assurance guidelines. Schepers et al. [18] demonstrated a reduction in the rate of contamination when appropriate procedures were in place.

We describe an outbreak of an aerobic actinomycete most closely related to *Microbacterium* species and *Plantibacter flavus*.

![Figure 2. PFGE findings for outbreak-associated isolates. Lane 1, λ Ladder; lanes 2–10, outbreak isolates from different transplant products; lane 11, Corynebacterium species isolated from the laminar flow hood.](image)

**Table 3. Summary of the microbiological culture survey of cryopreserved transplant products.**

<table>
<thead>
<tr>
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<tr>
<td>No. of transplant product samples cultured</td>
<td>229</td>
<td>73</td>
<td>86</td>
</tr>
<tr>
<td>No. of positive culture results</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before cryopreservation (culture point &quot;A&quot;)</td>
<td>0</td>
<td>2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>After cryopreservation (culture point &quot;B&quot;)</td>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>At the time of infusion (culture point &quot;C&quot;)</td>
<td>—</td>
<td>5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>—</td>
</tr>
</tbody>
</table>

<sup>a</sup> Culture positive for coagulase-negative staphylococci.

<sup>b</sup> Culture positive for the outbreak strain.
The taxonomy of this heterogeneous group of gram-positive rods continues to evolve, and it is difficult to assign the outbreak strain to a particular genus. These organisms are not traditionally considered to be human pathogens. Indeed, the growth characteristics of the outbreak strain suggest that it would be ill suited to colonize or infect humans. Although the outbreak strain was isolated from the blood sample of 1 patient, the isolation occurred soon after the contaminated transplant product was reinfused. It is possible that the organism was still present on the hub of the central line from which the culture sample was obtained, or that, if a true episode of bacteremia was present, it was not actually pathogenic.

Alonso-Echanove et al. [9] describe a nosocomial outbreak of Microbacterium bacteremia in patients with cancer at their institution. Eight bacteremic episodes occurred in 6 patients; all patients were symptomatic, and 1 patient died. Although the source of the Microbacterium species was not identified, it is speculated that it was a contaminated transplant product. Microbacterium species have also been reported to be a cause of endocarditis, central venous catheter–associated sepsis, surgical site infection, and endophthalmitis.

This outbreak was of point source origin and resulted from true blood product contamination rather than simple contamination of cultures. We hypothesize that the multiple-use bottles of Media 199 were the source of contamination, because the outbreak began after switching to the multiple-use bottles and stopped after use of filter sterilized single-dose media was re instituted. Outbreaks resulting from the contamination of multidose vials are well-known phenomena [19]. The fact that other bottles from the same lot number were culture negative makes the organism from the work surface.

The ultimate environmental source of the outbreak strain is unknown. The organism may have been intermittently present in the environment or present for a relatively short period of time. The laminar flow hood was operating at a reduced flow rate, which may have allowed for contamination of the work surface once the organism was introduced into the area. Appropriate cleaning protocols would have facilitated removal of the organism from the work surface.

In conclusion, we describe a outbreak of aerobic actinomycete contamination of transplant products. This outbreak was preventable in that proper technique and laboratory policies would likely have prevented contamination from occurring. However, even if contamination had been unavoidable, had appropriate quality assurance measures been in place to ensure that routine cultures had been performed after product manipulation and not before, the problem would have been identified before reinfusion, avoiding a potentially life-threatening situation.

References

16. Foundation for the Accreditation of Hematopoietic Cell Therapy (FAHCT). Standards for hematopoietic progenitor cell collection, pro-