Prevalence of bacterial contamination in blood and blood products at the National Blood Service Zimbabwe

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Abstract
Introduction: Advances in screening for infections improve the safety of donated blood. Transfusion-related bacterial sepsis, although not established in Zimbabwe, still makes bacterial contamination of blood clinically relevant.

Methodology: This cross-sectional study was conducted in Harare. Bacteriological and antibiotic susceptibility testing were done using standard methods.

Results: Of the 196 samples analyzed, 6 (3.1%) were contaminated with bacteria. Platelets had a significantly high contamination rate compared to other blood products. Bacteria showed varying patterns of susceptibility to the antibiotics tested.

Conclusions: The prevalence of bacterial contamination in blood products suggests that patients who receive blood products are at risk of developing infection.

Key words: bacterial; contamination; transfusion infections; Africa.


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Introduction
Technological advances in screening for transfusion transmissible infections (TTIs) such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), and syphilis have greatly improved the safety of donated blood. In Zimbabwe, these TTIs are routinely screened and diagnosis is aided by improved donor selection methods, and the risk of their transmission through blood transfusion is considered low. Bacterial transmission through blood transfusion has been identified as the cause of infectious complications associated with transfusion in various settings [1-5]. In the United States, bacterial contamination is considered to be the most prevalent infectious risk of blood transfusion and accounts for mortality rates ranging from 1:20,000 to 1:85,000 donor exposures each year [6]. The Food and Drug Agency (FDA) reported that 12% of all transfusion-related fatalities were caused by bacterial infections during the period 2005–2006 [7].

Approximately 57% of all TTIs and 16% of transfusion-related deaths have been associated with bacterial contamination [8]. Bacteria implicated include Staphylococcus aureus, Escherichia coli, and Staphylococcus epidermidis. In a study carried out in Ghana on bacterial contamination of blood products, whole blood accounted for the greatest percentage of contaminated blood products (13%), followed by platelets (9%) and plasma (3%) [9]. Gram-positive bacteria isolated were coagulase-negative Staphylococcus, S. aureus, and Bacillus spp., while Gram-negative bacteria included Yersinia enterocolitica, Citrobacter freundii, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae. These bacterial species have also been implicated in studies carried out in Nigeria, the United States, France, and Japan, clearly indicating that they are a real threat to blood supplies worldwide [10-13]. The prevalence of bacterial contamination and the clinical implications of contaminated blood and its products in Zimbabwe are not yet known.
Several studies on bacterial contamination of blood products have reported platelets as the most commonly contaminated blood product [3,5,6,14-16]. In the United States, platelets accounted for the most common transfusion-associated infectious risk; they were reported to cause a 50- to 250-fold increase in the risk of transmission of HIV, HBV, and HCV [14]. Bacterial contamination is estimated to occur at an incidence of 1:1,000 to 1:3,000 in platelet units, with severe episodes estimated to occur in about one-sixth of contaminated products [12].

Bacterial contamination of blood components has been tremendously reduced in developed countries through the use of various techniques. Phlebotomy sites for blood donors are carefully prepared using improved skin disinfection methods. Blood components, particularly platelets, are cultured using a rapid detection system; for example, in 2006, the United States FDA gave formal recognition of the Pall e-BD system for rapid detection of bacteria in blood and platelets [15]. Diversion of the first aliquot of donor blood and pathogen inactivation with ultraviolet rays has been used in the United States to reduce the risk of bacterial transmission.

In contrast, some of these techniques that are scientifically proven to reduce risk of bacterial contamination are not available in Zimbabwe. Although the prevalence of transfusion-related bacterial sepsis has not been established in this country, reports on transfusion reactions suggest that prevalence of bacterial contamination of blood components might be of clinical relevance [17]. Taking into consideration the high demand for blood products and high prevalence of infections among the Zimbabwean population, this investigation aimed to estimate the prevalence of bacterial contamination in blood products, to identify bacterial species contaminating blood products, and to identify blood products most likely to be contaminated with bacteria.

**Methodology**

**Study site and study**

This was a single-site cross-sectional study conducted in Harare, Zimbabwe, between April and August 2013. Random sampling of stored blood products (packed cells and platelets) meant for transfusion was done for all samples included in the study. Fresh frozen plasma and cryoprecipitate were excluded from the study as they could not be re-used after thawing and refreezing. Samples from blood products that had tested positive for routinely tested TTIs (HIV, HBV, HCV, and syphilis) were also excluded. Ethical approval to carry out the study was granted from the Joint Parirenyatwa Hospital Research Ethics committee and National Blood Service Zimbabwe.

**Blood and blood product collection**

Sample processing was done using standard bacteriological safety and aseptic techniques. Disinfection was done using 70% ethanol. Stored blood and blood products were thoroughly mixed, and the end of the tied tubing was swabbed, disinfected, and cut with sterile scissors to discard clotted blood in the line. Some of the mixed blood from the main bag was allowed to seep into the line. The end of each line was clipped with sterile forceps to prevent blood from flowing back into the main bag. Two knots were made on the line, and the last knot was swabbed with 70% ethanol and punctured with a sterile needle and syringe to draw 10 mL of blood product. The samples were dispensed into separate, sterile bottles containing 50 mL of trypton soy broth.

**Culturing and identification**

All of the suspensions were incubated aerobically at 37°C for up to seven days and observed for signs of bacterial growth on days 2 and 7. For samples showing signs of bacterial growth, a Gram smear was made and examined microscopically. At the same time, the samples were subcultured using standard methods onto blood agar (BA), chocolate agar (CA), and MacConkey agar (MA). Blood agar and MA plates were incubated in air, while CA plates were incubated in candle jars at 37°C for up to 48 hours. Plates were inspected for bacterial growth at 24 hours (BA, CA, and MA) and 48 hours (BA and CA). Further microbiological identification of isolates was done using standard biochemical and sugar fermentation methods.

**Statistical analysis**

The Epi-Info Statistical software package was used to analyse the data. The Chi-square test was used to test any statistically significant differences between the data. Any differences where p < 0.05 were considered statistically significant.

**Results**

A total of 196 samples (149 packed cells, 39 platelets, and 8 whole blood) were randomly collected and cultured. Of the 196 samples, 6 (3.1%) samples were found to be contaminated with various species of bacteria. Platelets had a significantly higher level (p <
0.05) of bacterial contamination compared to other blood products (packed cells and whole blood). The different levels of contamination of the blood products are summarized in Table 1.

From all blood products sampled, platelets had the highest prevalence of bacterial contamination; 10.3% Gram-positive bacteria (S. aureus, coagulase-negative \textit{Staphylococci}, and \textit{Bacillus} sp.) and Gram-negative bacteria (\textit{E. coli}) were isolated, accounting for 83% and 17% of the platelet samples, respectively.

Of the contaminated platelets, one (25%) was at storage day 4, while three (75%) were at storage day 5. Of the contaminated packed cells, one was at day 21 of storage, while the other was at day 32 of storage.

Table 1. Levels of contamination in blood products

<table>
<thead>
<tr>
<th>Blood Product</th>
<th>No. of blood packs tested</th>
<th>No. of blood packs contaminated (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Packed cells</td>
<td>149</td>
<td>2 (1.3)</td>
</tr>
<tr>
<td>Whole blood</td>
<td>8</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Platelets</td>
<td>39</td>
<td>4 (10.3)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>196</strong></td>
<td><strong>6 (3.1)</strong></td>
</tr>
</tbody>
</table>

Discussion

Bacterial contamination of blood products remains a very critical transfusion related risk often ignored in Africa. Knowledge of the prevalence of bacterial contamination in blood products and their source is important for the planning of prevention and reduction measures that reduce mortality and morbidity arising from transfusion of contaminated blood products. The majority of blood component recipients are children and women in Africa; reducing the mortality in this group will depend on access to safe blood [1].

This study determined an overall bacterial contamination prevalence of 3.1%. Studies done in other parts of Africa (Ghana, Nigeria, and Ethiopia) found higher prevalence rates of 9%, 8.8%, and 12.5%, respectively [9,10,18]. However, the prevalence of bacterial contamination is reported to be lower in developed countries, with prevalence rates of 0.2% in the US and 0.1% in France [11,12]. The isolation of Gram-negative bacteria such as \textit{E. coli} is of concern, as it has been reported that patients who are at greatest risk of death were those who had received blood units contaminated by Gram-negative bacteria [12].

In the present study, platelets had the highest prevalence of bacterial contamination than did any other product sampled. These findings are consistent with other studies done in other countries, including Japan [11], the United States [12], and France [13]. It is of public health importance to include rapid bacterial testing for all platelets before they can be issued for transfusion. Blood transfusion centers must adopt active surveillance methods for detecting platelet contamination to improve the safety of transfusions [5]. Platelets are stored between 22 and 24°C with constant agitation, which is favorable for bacterial proliferation.

Our study was limited because the source of bacterial contamination could not be established. Donor bacteraemia, inappropriate disinfection of venepuncture site, contaminated blood bags, or poor storage conditions could be the sources of bacterial contamination. Further studies are necessary to identify the potential sources of bacterial contamination in blood products and to adopt measures to avoid future contamination. There is also a need to explore and assess the effectiveness of other techniques proven to reduce bacterial contamination of blood, including leuko-depletion, pathogen inactivation, and diversion of the first aliquots of blood, in Zimbabwe.

The clinical outcomes from transfusion of contaminated blood products vary based on the type of implicated bacteria, the immune status of the blood component recipient, and antimicrobial therapy at the time of transfusion [3,6]. Zimbabwe has a high burden of immunosuppressive illnesses, including acquired immune deficiency syndrome (AIDS), chronic cancers, and diabetes, and therefore the potential adverse effects of contaminated blood products cannot be overlooked. This study did not follow up recipients of contaminated blood products, and so patient outcomes from transfusion of contaminated blood could not be ascertained. Further studies on the clinical outcomes following transfusion of contaminated blood products are necessary.

The results suggest that the prevalence of bacterial contamination in blood products in Harare, Zimbabwe, was high, and patients who received blood products...
(especially platelets) are at risk of developing infection. The prevalence of bacterial contamination in Harare suggests the urgent need for the introduction of a wide range of bacterial reduction techniques, especially in the developing world.

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