Annals of Medical Laboratory Science
Volume 3, Number 1, Page 12-21 June 2024
https://doi.org/10.51374/annalsmls.2024.3.1.0004
ISSN: 2805-4024

Bacterial Contaminants in Stored Blood and Blood Products at Zomba Central Hospital Blood Bank: Assessing the Possible Risk of Post-Transfusion Sepsis in a Resource-Limited Setting

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Received 19-02-2024
Accepted 30-04-2024
Published 30-06-2024

Abstract

Background: National Blood Transfusion Services have done a commendable job in reducing transfusion-related fatalities from viral, Syphilis, and Malaria infections through the vigilant screening of blood donors and donated blood. Transfusion of bacterially contaminated blood and blood products remains the commonest cause of transfusion-associated fatalities, but it remains unaddressed in resource-limited countries. Without hemovigilance programs in countries like Malawi, up-to-date knowledge of the prevalence and causes of bacterial contamination of blood products is necessary to ensure safe blood transfusion. This study investigated the rate and spectrum of bacterial contaminants in stored blood and blood at the Zomba Central Hospital from October to November 2022.

Methods: 115 blood products (Whole Blood, Packed Red Blood Cells, and Platelets) were randomly and aseptically collected into Tryptic Soy Broth and then incubated for 7 days. After overnight incubation, all samples were subcultured onto Blood Agar, Chocolate Agar, and MacConkey Agar. Colony morphology, gram staining reactivity, and biochemical tests were used to identify the isolated organisms. The data was analyzed using correlation and regression statistics, and results with $p \leq 0.05$ were considered significant.

Findings: Of the 115 samples, 21 (18.3%) were contaminated with gram-positive bacteria. The contaminants were Bacillus spp (33.33%), Listeria spp (33.33%), Coagulase-negative Staphylococcus (19.05%), Staphylococcus aureus (9.52%), and Enterococcus spp (4.76%). 90.5% of all the contaminated products had exceeded 2 storage weeks.

Conclusion: Bacterial contamination of stored blood products is common at the study site. This study emphasizes the need to implement hemovigilance projects to reduce the risk of post-transfusion sepsis in resource-limited settings.

Keywords: Bacterial contamination, sepsis, transfusion-transmitted infection, blood transfusion.

INTRODUCTION

Blood transfusion is a medical practice that has served an important role in saving lives and improving the quality of life in a variety of clinical conditions for the past few centuries (1). However, whole blood and blood components for transfusion can be a source of transfusion-transmitted infections (TTIs) from viral, bacterial, and parasitic pathological agents (2, 3). Recently, much emphasis has been directed toward minimizing the risk of transmission of viruses, Treponema pallidum, and Plasmodium species through blood transfusion. Because of the high prevalence of immunosuppressive diseases like HIV in Africa, the low but definite risk of bacterial contamination continues to pose a significant threat to the safety of blood transfusion (4-6).

Bacterial infection through blood may lead to complications like sepsis, pneumonia, abscesses, wound infection, meningitis, haemolysis, empyema, urinary tract infection, and fever (7, 8). In developed countries, 57% of all TTIs and a mortality of 16% from all TTIs have been attributed to bacterial contamination of blood and blood components (9). Even after taking various precautions during blood collection and processing, bacterial contamination still takes place through various endogenous and exogenous means (10). Bacterial inoculation into blood bags may result from insufficient disinfection of the venepuncture site, already existing asymptomatic bacteraemia from the donor, contamination from the environment, or handling blood during blood component preparation (11).

Another factor that is overlooked is the storage conditions of these blood products. With the frequent power outages in countries like Malawi, there are likely to be temperature fluctuations in the blood banks, which may facilitate the proliferation of bacteria. Most of the preventive actions currently implemented by developed countries are not yet accessible in developing countries, including Malawi (12). For developed countries like the United States of America, France, and the United Kingdom, studies report that bacterial contamination of donated blood is at 0.2%, 0.1%, and 0.15%, respectively. (10)The numbers are higher in African studies, however, with rates estimated to be between 8% and 18%, according to Onchaga et al. (13).

The organisms most commonly isolated from contaminated blood and blood components include normal flora from the skin and bacterial contaminants from the environment. Predominantly, Gram-positive organisms, including Bacillus species, Streptococcus species, Staphylococcus species, and Gram-negatives like Yersinia enterocolitica, Pseudomonas fluorescens, Pseudomonas aeruginosa, Salmonella species, Escherichia coli have all been implicated (9, 13, 14). Bacterial contamination is a leading cause of transfusion-related deaths after ABO-mismatch (15).

Various improvements in donor screening, blood collection and pre-transfusion testing have helped in deflating transfusion-transmitted viral infections to less than one in a million blood transfusions (16, 17). In contrast, there has been at least one culture-confirmed post-transfusion sepsis case out of 100,000 recipients, with a fatality of 1 in 500,000 recipients (13). Procedures like leukocyte depletion, diversion methods, and pre-donation sampling can help to minimize transfusion-related bacterial infections from contaminated blood and blood products (18). However, most of these methods are inaccessible in developing countries, including Malawi.

Though sporadic, the data collected within Sub-Saharan Africa estimated bacterial contamination ranging from 8.8% to 17.5% (13). With the rapid emergence of antimicrobial-resistant bacteria, septicaemia can be prevented by providing safe blood and blood products, which can reduce mortalities if taken seriously. Although transfusion-associated bacterial sepsis has been given insufficient acknowledgment in Malawi, it remains a serious public health matter. This study, which was conducted at ZCH, assessed the bacterial contamination of stored blood and blood products collected and prepared by the Malawi Blood Transfusion Services.

METHODS

This study used a laboratory-based cross-sectional study design with a quantitative application to demonstrate the bacterial contamination of blood and blood products. It was conducted at ZCH, one of the referral centers in Malawi. A total of 115 samples were collected from Whole Blood, Packed Red Cell concentrates, and Platelet concentrates.

Sample Collection and Culturing

Samples were collected from the portions at the end of the satellite tube linings with 3 to 4 of the portions per blood unit sampled. After thoroughly disinfecting the tube portions with 70% isopropyl and 2% tincture of iodine, a pair of sterilized scissors was used to cut the two ends of the portions. Then, the blood was left to flow into

the pre-enrichment culture media. The procedure was conducted in a well-disinfected biosafety cabinet to prevent contamination of the samples. All broth suspensions were incubated aerobically at 37°C. After overnight incubation, a loopful of every sample in the enrichment media was then sub-cultured onto Blood Agar, Chocolate Agar, and MacConkey Agar according to the Standard Operating Procedures. Chocolate Agar plates were incubated anaerobically.

Identification of Bacterial Isolates

For Species identification, colonial morphology description was employed, followed by a gram-stain test and necessary standard biochemical tests for further identification of the bacterial isolates.

Data Analysis

Data recorded in Microsoft Excel 2016 was cleaned and analyzed using the Statistical Package for Social Sciences (SPSS) software version 22.0 (IBM, USA). Descriptive statistics, Linear regression Correlation statistics, and two-sampled t-tests were computed to analyze the study findings. Standard deviation and Mean were employed to present the distribution of bacterial contamination among the blood components. Correlation and Regression statistics were calculated to analyze the relationship between storage days and the frequency of contamination. Findings were regarded as significant if $p \leq 0.05$. The data was also presented in tables and charts with frequencies and proportions at 95% confidence interval.

Ethical Considerations

Ethical approval was acquired from the Mzuzu University Research Review Committee under the Health Sciences Faculty Research Committee (Ethical Clearance number: FOHS/REC/21/202). An approval letter from the ZCH Research Committee was also obtained for permission to conduct the study at the ZCH Laboratory.

RESULTS

Table 1 shows the blood products sampled in this study. Out of the 115 blood products, the majority of the units were Whole Blood (n=57, 49.6%), followed by Packed Red Cell units (n=52, 45.2%) and Platelet units (n=6, 5.2%). There were 61 blood products of blood group O RhD Positive, representing 53.0%, then 25 (21.7%) units of blood group A RhD Positive, 24 (20.9%) units of blood group B RhD Positive, and 5 (4.3%) units of blood group AB RhD Positive. The sample did not contain Rh-negative products. The storage duration of the blood products ranged from 4 to 40 days. Additionally, the average storage days of the sampled blood products was 15.10 with a standard deviation of 8.19 days.

The overall prevalence of bacterial contamination was 18.3%, as 21 of the 115 blood product samples were found to be contaminated with bacteria, while 94 (81.7%) showed no bacterial growth after 48 hours of incubation (Figure 2). In this study, all 21 organisms isolated from the blood products were Gram-positive bacteria. The majority of the isolates included Bacillus species (33.33%) and Listeria species (33.33%) (n=7), while 19.05% were Coagulase-Negative Staphylococcus (n=4), followed by 2 Staphylococcus aureus isolates (9.52%) and 1 Enterococcus bacteria representing 4.76% (Figure 1). The possible sources of the bacterial contaminants include donor skin, donor blood, and the environment, mostly the soil and water. Of the isolated organisms, 71.36% are known environmental bacteria, and 38.09% are skin-normal flora. Gram-negative species were not isolated in this study.

Platelets had a higher contamination rate (33.3%, n=2/6) than the rest of the products. Of the 52 Packed RBCs, 11 and 8/57 of Whole Blood units were contaminated. According to Phi and Cramer's V correlation statistics, but there is no significant association between the type of blood product and the levels of bacterial contamination ($p = 0.389$). Out of the 21 contaminants isolated, most were from the Blood Group O RhD Positive (n=12). Blood Group A RhD Positive had 4 contaminants isolated. 3 and

2 contaminants were isolated from Blood Groups B RhD Positive and AB RhD Positive, respectively. There was no significant association between the blood group and the bacterial contamination of the blood products ($p = 0.516$).

There was no significant difference ($p > 0.567$) in the means of the storage days between the blood products and those not contaminated after using the two-sample t-test. The blood units with bacterial contamination had an average storage period of 14.18 days as compared to those without contamination (Mean=15.32) (Table 2). The highest bacterial growth rate was observed in blood products with 2 weeks of storage (n=10). Storage week 3 registered 7 contaminants, while weeks 1 and 4 had 2 contaminants each. All the blood products beyond 4 storage weeks had no bacterial contamination. About 90.5% of the contaminated samples had more than 2 weeks of storage. Linear regression statistics ($R = -0.457$, $p = 0.330$) calculated to analyze the relationship between the storage duration and the number of contaminants isolated revealed no significant association between the two variables (Table 3). However, a declining trend was observed on the line chart between the frequency of contamination and the storage weeks (Figure 2).

Table 1: Characteristics of the blood products (N=115)

Characteristics		
Type of Blood Components		
Whole Blood *N* (%)	57	49.6
Packed Red Cells *N* (%)	52	45.2
Platelets *N* (%)	6	5.2
Blood Groups of Blood Components		
A Rh D Positive *N* (%)	25	21.7
B Rh D Positive *N* (%)	24	20.9
AB Rh D Positive *N* (%)	5	4.3
O Rh D Positive *N* (%)	61	53
Statistics for Storage Days		
Minimum	4	-
Maximum	40	-
Mean	15.1	-
Standard Deviation	8.19	-
N	115	-

Table 2: Summary Statistics for Storage Days of Products

	Mean (Days)	**SD**	**Median**
No Growth	15.32	8.648	12.00
Growth	14.18	5.949	14.00
Total	15.10	8.190	12.00

Table 3: Regression Statistics for Storage Weeks and

Model	**R**	**R Square**	***p*-value**
1	-0.457	0.209	0.330

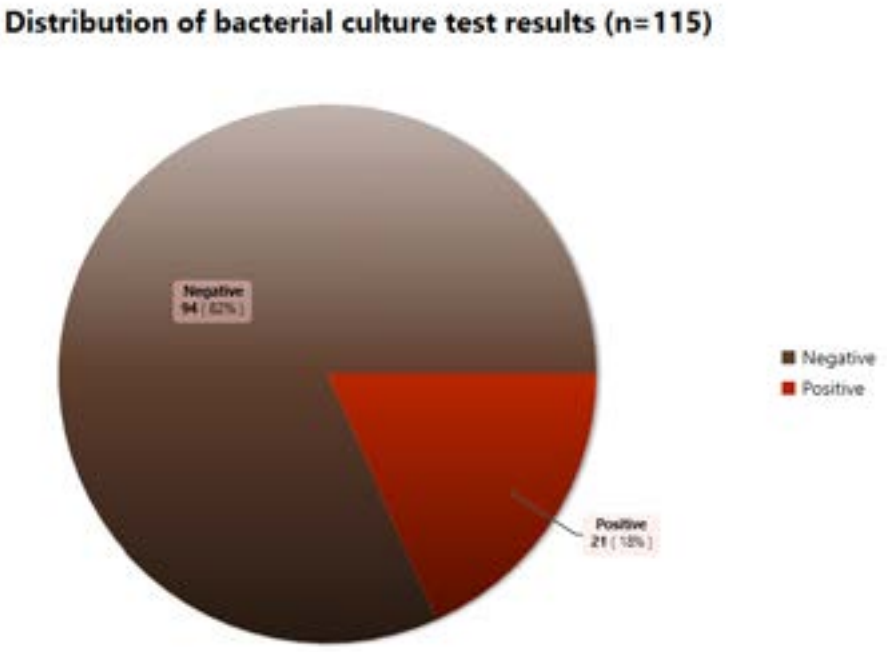

Figure 1. The Distribution of bacteria culture results. 94/115 (81.7%) of the units were culture-negative and 21/115 (18.3%) were culture-positive.

Table 1. Possible contamination sources of the isolated bacteria

Species	N. of isolates (%)	Possible Source
Staphylococcus aureus	2 (9.52%)	Donor Blood & Skin
Listeria spp	7 (33.3%)*	Environment
Bacillus spp	7 (33.3%)*	Environment
Coagulase-Negative staphylococcus spp	4 (19.05%)	Donor Skin
Enterococcus spp	1 (4.76%)	Environment

The possible sources of the bacterial contaminants include donor skin, donor blood and the environment mostly the soil and water. Table 2 shows that 71.36% are known environmental bacteria, and 38.09% are skin normal flora (Table 1).

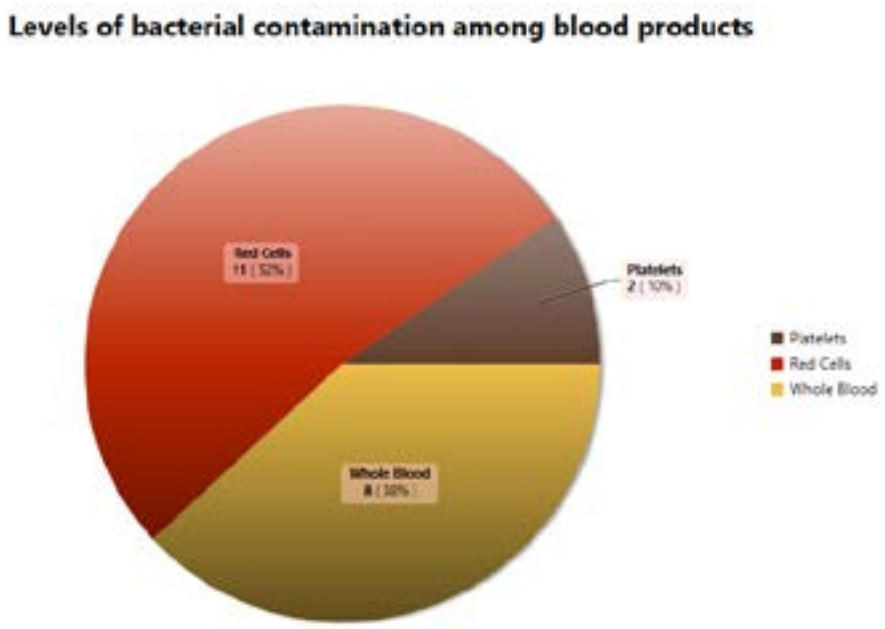

Figure 2. Levels of bacterial contamination among the blood products.

Platelets had a higher contamination rate (33.3%, n=2/6) than the rest of the products. Of the 52 Packed RBCs, 11 were contaminated, as were 8/57 of Whole Blood units (Figure 2).

According to Phi and Cramer's V correlation statistics, but there is no significant association between the type of blood product and the levels of bacterial contamination (P = 0.389) (Table 2).

Table 2. Correlation statistics for blood products and the level of contamination

		Value	**P Value**
Nominal by Nominal	Phi	0.128	0.389
	Cramer's V	0.128	0.389

Out of the 21 contaminants isolated, most were from the Blood Group O RhD Positive (n=12). Blood Group A RhD Positive had 4 contaminants isolated. 3 and 2 contaminants were isolated from Blood Groups B RhD Positive and AB RhD Positive, respectively (Table 3).

Table 3. Levels of contamination among blood groups

	Culture Results		
Blood Group	**Negative (%)**	**Positive (%)**	**Total**
A RhD Positive	21 (84.0)	4 (16.0)	25
B RhD Positive	21 (87.5)	3 (12.5)	24
AB RhD Positive	3 (60.0)	2 (40.0)	5
O RhD Positive	49 (80.3)	12 (19.7)	61

DISCUSSION

Prevalence of Bacterial Contaminants in Blood and Blood Products

In this study, the prevalence of bacterial contamination of blood and its components was 18.3% (n= 21/115). This finding is marginally higher following studies done in Egypt (17.9%) (19), and Ghana (16.5%) (12). This prevalence, however, is significantly higher when compared to studies from high-income countries like the United States of America (0.2%), the United Kingdom (0.15%) and France (0.1%) (10). The wide difference could be because low-income countries like Malawi do not use various preventive measures, such as diversion methods, inactivation of pathogens using ultraviolet rays, pre-donation sampling, double disinfection of the phlebotomy site, and the lack of national hemovigilance programmes.

Tsegaye et al. (20) compared diverging and non-diverging blood collection methods and reported that diverting the first 30-40 ml of blood during collection reduced the bacterial contamination rate by 5.8% in their study. It is also important to note that there were frequent power outages and a lack of backup power within the months of September and October. Consequently, the blood products were exposed to temperatures greater than 8°C for considerable periods. This might have contributed to the high prevalence of bacterial contaminants as the bacteria would have been able to survive and proliferate for longer periods.

Bacteria Species Isolated in the Blood and Blood Products

The majority of the isolated organisms were both Bacillus spp and Listeria spp (33.3%, n=7) followed by Coagulase-negative Staphylococcus (19.05%, n=4), Staphylococcus aureus (9.52%, n=2) and Enterococcus spp (4.76%, n=1). Only Staphylococcus aureus could have originated from donor blood out of these isolates. This could suggest that healthcare workers are vigilant in the comprehensive screening and recruitment of donors. However, the high prevalence of Gram-positive bacteria indicates poor scrubbing of the phlebotomy site during blood collection. Similarly, studies conducted in Uganda and India did not report Gram-negative bacteria in their findings. (1, 2).

In most of the studies that reported Gram-negative bacteria, Gram-positive bacteria still had the highest prevalence among the isolates. For instance, from the isolated bacteria, 80%, 77.8%, 82.4% 88.9%, 56.2%, and 79% were gram-positives (6, 10, 12, 13, 15, 18, 20). These findings align with my findings and point towards improper disinfection of the venepuncture site as the commonest source of bacterial contamination of blood and blood components. Nevertheless, the predominant isolation of Gram-positive bacteria does not reduce the possibility and severity of post-transfusion sepsis as Staphylococcus spp and Bacillus spp have been reported to cause most cases. (4, 21).

Most of the isolated bacteria are commonly found on human skin, blood, and the environment. Staphylococcus spp are normal skin flora, but Staphylococcus aureus has also been isolated in many cases of bacteraemia. (21)Bacillus, Enterococcus, and Listeria spp are readily found in water and soil. Therefore, the possible sources of the organisms isolated in this study may have included dust within blood donation areas, contaminated cold chain boxes, poor skin disinfection during phlebotomy, and donor-asymptomatic bacteraemia.

The high prevalence of bacterial contaminants found in this study suggests that blood transfusion in Malawi poses a great risk of transfusion-related bacteraemia. However, since Gram-negative bacteria were not isolated, the infections from the isolated Gram-positive bacteria may not be very severe for immunocompetent individuals. Nevertheless, with the current emergence of antibiotic-resistant bacteria, the increased rates of HIV infection, and non-communicable diseases that lead to immunodeficiency, there have been reports of severe cases of sepsis and its complications caused by some of the gram-positive species isolated in this study. (21-25). Whilst bacteraemia

from Enterococcus spp is extremely rare, it has also been reported to have a high mortality rate when treatment is delayed. (26). Therefore, the Gram-positive bacteria isolated in this dissertation still pose a great risk to blood transfusion safety in Malawi.

Distribution of Contamination among Blood and Blood Products

In this study, Packed RBCs were the most contaminated blood product, with 11 (52%) units contaminated, followed by Whole Blood (n=8, 38%) and platelets (n=2, 10%). These findings were not in agreement with the findings by Sharma et al., (14) and Makuni et al., (9). However, the results are biased as the blood products sampled were not of equal proportions. Tsegaye et al. (20) conducted a similar study but with equal samples of Whole Blood, Packed RBCs, Platelets, and Fresh Frozen Plasma. The findings revealed that Whole Blood and Packed RBCs had a higher bacterial contamination rate than Platelets. The correlation statistics calculated showed that there was no significant relationship between the type of blood products and the rate of bacterial contamination ($p = 0.389$).

Another study with a larger sample size and equal proportions of blood products needs to be conducted to assess further the difference in the risk of post-transfusion sepsis from different blood products. Although platelets have been reported to have the highest risk of bacterial contamination given their storage conditions among the blood products, this study suggests that all blood products have an equal probability of causing transfusion-related sepsis. There was no significant association between the blood group and the bacterial contamination of the blood products ($p = 0.516$). Unlike these findings, various studies reported a high contamination rate for blood group O RhD Positive. (2, 14, 20).

The Storage Duration and the Frequency of Bacterial Contamination

Literature suggests that bacteria require a period of proliferation in storage before they can be detected and that most of the contaminants are isolated within the first week of storage. This is because the survival and proliferation of bacteria is reduced once the organism enters the blood bag due to proper cold chain storage and limited blood and blood products storage. On the contrary, the present study revealed that 90.5% of the contaminated blood and blood products had exceeded 2 weeks of storage. These results are not in agreement with the study conducted in India, where 76.46% of the contaminated units had less than 1 week of storage (14).

Agzie et al. (10) A study conducted by Heroes et al. also reported that blood units stored for 0 to 3 days had the highest contamination rate. (6), states that bacterial contamination was higher in older blood and blood products than the products, although the days and weeks were not specified. The reasons for the variations cannot be ascertained from this study. However, it is important to note that ZCH Laboratory had a few challenges that may have helped explain these findings. It was noted that the refrigerator used to store these units was frequently opened during working hours, which might have compromised the cold chain conditions. In addition, there were frequent power failures coupled with the lack of backup power from the hospital generator due to the fuel crisis in the country at the time. All these could have contributed to the longevity of the bacteria in the blood products as they were exposed to temperatures higher than 8°C for long periods.

There seemed to be a decreasing trend in the rate of bacterial contamination as the storage weeks increased, as portrayed by Figure 2, indicating that even with poor storage conditions, bacteria cannot survive past a specific period in storage. Nevertheless, there was no significant relationship ($p > 0.05$) between the storage period and the rate of bacterial contamination of blood products. A study from Ethiopia had a similar conclusion (2) Thus, we can hypothesize that the rate of bacterial contamination depends not only on the storage period but also on the conditions in which the blood products are kept. Ambient and refrigeration temperatures must be constantly monitored with

well-calibrated thermometers to ensure proper room and cold chain conditions and to reduce the survival and proliferation of bacteria in various blood components.

The overall prevalence of bacterial contamination was 18.3%, as 21 of the 115 blood product samples were found to be contaminated with bacteria, while 94 (81.7%) showed no bacterial growth after 48 hours of incubation (Figure 1).

CONCLUSION

This study revealed that the prevalence of bacterial contamination of stored blood and blood products at ZCH was higher than in various studies conducted in other countries. All isolated organisms were gram-positives, hinting at poor disinfection techniques during the blood donation process at the phlebotomy site. Bacillus and Listeria spp were the most isolated bacteria, with Coagulase-negative Staphylococcus, Enterococcus spp, and S. aureus also identified. There was no significant relationship between the blood products' bacterial contamination rate and the type of blood product, the blood group, or the storage period ($p > 0.05$).

Since the study only included blood and blood products from one blood donation centre (Blantyre), the findings may not be used to represent the rest of the blood collection centres (Balaka, Lilongwe, and Mzuzu). The study also did not include follow-up of blood recipients for clinical outcomes to check if they developed any kind of septicaemia. Finally, the study could not establish the source of the bacterial contaminants. Therefore, the possible sources elaborated in this report are purely based on commonly established habitats of the isolated bacteria.

In conclusion, there is a need for serious coordination between the Ministry of Health, the MBTS, and all stakeholders in the blood transfusion process to counter this silent threat. The establishment of national hemovigilance programmes is highly recommended, which can promote the introduction of blood donation bags with diversion pouches and periodic training of healthcare workers on the blood transfusion process, including awareness of transfusion-related sepsis. Lastly, there is a need to enforce improved donor arm disinfection using 70% Isopropyl alcohol and then 2% Chlorhexidine as recommended by the National Health Services in England (27).

ABBREVIATIONS

RBC Red Blood Cells
Spp Species
ZCH Zomba Central Hospital
MBTS Malawi Blood Transfusion Services

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How to cite this paper: Office EA, Salimu TC. Bacterial Contaminants in Stored Blood and Blood Products at Zomba Central Hospital Blood Bank: Assessing the Possible Risk of Post-Transfusion Sepsis in A Resource-Limited Setting. *Annals of Medical Laboratory Science 2024*; 3(1): 12-21