

Bacterial contamination of blood and blood components in a tertiary hospital setting in Nigeria

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Abstract

Screening donors has practically eliminated viral pathogens in blood for transfusion; however, transfusion-associated bacterial sepsis remains an important health-care concern. Currently, it is the most frequently reported cause of transfusion-related fatality from infection. The aim of this study was to determine the prevalence and type of bacterial contamination in donor blood and/or blood products, in a semi-urban university teaching hospital in Nigeria.

The study was carried out at the Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria over a five-month period (May to September 2009). A total of 162 units of screened blood and blood products consisting of 160 (98.7%) refrigerated packed cells/whole blood and two (1.3%) platelet concentrates were randomly sampled following aseptic procedure. Samples were incubated at 37°C for up to 7-days in Brain Heart Infusion broth. Isolates were identified by standard microbiologic techniques, and their resistance to selected antimicrobial agents was tested by disc diffusion.

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Overall prevalence of bacterial contamination in donated blood bank refrigerated blood was 8.8%. The organisms isolated were Gram-positive, namely, *Staphylococcus aureus*, coagulase negative *Staphylococcus*, *Bacillus* sp, and *Listeria* sp. Antibiotic resistances ranged from 29% to 100%.

Bacterial contamination of blood and blood components for transfusion is common in Nigeria and is a potential risk for hospital acquired infection.

Key words

Bacterial contaminants, hospital-acquired infection, blood/blood components.

Introduction

The transmission of infectious agents including bacterial and viral organisms through blood and blood products has been long established. Recipients of donor blood have been known to acquire human immunodeficiency virus (HIV), Hepatitis B virus (HBV) and Hepatitis C virus (HCV), human T-cell lymphotropic virus (HTLV), *Treponema pallidum* and *Mycobacterium tuberculosis* through blood transfusion;^{1,2} while cytomegalovirus (CMV) infection is a serious risk in bone marrow and organ transplant patients.³ Over the years, improved donor screening almost eliminated HIV, HBV and HCV from the United States blood supply.⁴ In Nigeria like other developing countries, these transfusion-transmissible infections still pose a threat; several reports have documented transmissible pathogens (HIV, HBV, HCV, and *Treponema pallidum*) in blood donors.^{5,6} Considerable efforts (and national policies) directed towards reducing transmissible pathogens (improved donor selection and newer screening techniques) have yielded a major reduction of these agents in advanced countries.⁷⁻⁹ However transfusion-transmitted bacterial infection was identified as the commonest cause of complications associated with transfusion,¹⁰ numerous studies have demonstrated that contaminating bacteria (usually skin flora) can be cultured from approximately 1/3000 platelet units.¹¹ According to data from SHOT (Serious Hazards Of Transfusion), the haemovigilance system in the UK, in 1995-2006, there were 33 cases of post-transfusion bacterial sepsis, 29 due to transfusion of platelets and four to red cell concentrates. No deaths were caused by Hepatitis viruses, HIV or HTLV-I. Eight deaths were caused by bacterial contamination of transfused

platelets. In the United States, bacterial contamination of blood and blood products accounted for 15.9% of all transfusion related fatalities, indeed it is considered the second commonest cause of death from transfusion after clerical errors.¹² In Ghana, 9-17.5% of donor bloods were contaminated by bacteria.^{13,14} The bacteria transmissible in blood and blood components include Gram negative bacteria- *Klebsiella pneumoniae*, *Escherichia coli*, *Yersinia enterocolitica*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa* and Gram positive bacteria including *Bacillus species* and *Staphylococcus aureus*.¹³⁻¹⁴ Arguably, bacterial contamination of whole blood and its various components can occur at several points including production of blood bags, donor venepuncture, blood donor bacteraemia, blood component separation or at the time of transfusion.¹⁵

In Nigeria, there are no reports of bacterial contamination of blood and blood products, despite the high demand for these created by children with severe anaemia secondary to malaria, victims of road traffic accidents or in obstetrics emergencies amongst many other indications. We hypothesized that bacterial contamination of donor blood is a significant but overlooked health hazard that may lead to hospital-acquired infection in recipients. Thus, we sought to determine the prevalence of bacterial contamination in our donor blood and blood components, identify the organisms involved and determine the antibiotic susceptibility pattern of any isolated microorganisms with a view to instituting appropriate infection control and preventive interventions.

Materials and methods

Study site and design

This was a cross-sectional study carried out at the Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ile-Ife, over a five month period (May-September 2009). OAUTHC is a 600-bedded tertiary hospital centre affiliated to the Obafemi Awolowo University located in a town of about 750,000 in Southwest Nigeria, and provides health care services to people from five surrounding states and referrals from other parts of the country. Each year about 3000 bags of whole blood are donated. 51.7% constitute family replacement donation and only 2.5% come from voluntary non-remunerated donors. The remainder (45.8%) is from paid commercial donors. All donors are screened for HIV, HBV, HCV and syphilis before blood collection. Blood collection is anchored by a trained blood donor organizer supported by phlebotomists dedicated to the bleeding room. Blood/blood components are collected in citrate-phosphate-dextrose-adenine (CPD-A) blood-bags after disinfecting the phlebotomy site with 70% ethanol following a standard procedure including hand hygiene.

Sampling

We randomly sampled 162 units representing 5% of annual donations. The blood samples were obtained from screened, stored whole blood, packed cells, fresh frozen plasma (ready for use) and platelet concentrate. The procedures were undertaken with attention to aseptic and safety precautions by one of us (RAB), who is a consultant Haematologist. All expired blood and blood products were excluded. Each unit of blood was mixed before sampling and the tubing was cleaned with 70% alcohol and cut with sterile scissors to remove any clotted blood and also allowing some mixed blood to slip out of the blood bag. Three knots were made in each bag tubing and re-cleaned with 70% ethanol; 3mls of blood was drawn from the closest end to the bag with a sterile syringe and needle and dispensed into 15mls of Brain-Heart Infusion (BHI) broth. The specimens were sent to the Microbiology laboratory for isolation, identification and susceptibility testing.

Bacterial isolation and identification

The broths were incubated at 37°C up to 7 days before they were discarded. After overnight incubation, sterile loopfuls of broth were sub-cultured on to blood agar and MacConkey agar plates and incubated aerobically for 18-24 hours at 37°C. The identities of bacteria growing on the culture plates were determined by colonial morphology, Gram and spore stains; as well as standard biochemical tests.

Antibiotic susceptibility testing

Susceptibility to antimicrobial agents was tested by the disc diffusion technique according to the guidelines by the Clinical and Laboratory Standards Institute (CLSI).¹⁶ The antibiotic discs used were ampicillin 10 µg; cotrimoxazole, 25 µg; erythromycin 15 µg; penicillin 10 units; tetracycline 30 µg; ciprofloxacin, 5 µg; gentamicin, 10 µg; ceftriaxone 30 µg (Remel, Lenexa, Kansas) and rifampicin 5 µg (Oxoid). The discs were placed on to the surface of inoculated Mueller-Hinton agar plates by an auto dispenser (Remel). After overnight incubation, the inhibition zone diameters were measured to the nearest millimeter, and isolates were classified as susceptible, intermediate, or resistant according to CLSI-specified interpretive criteria. Intermediate and resistant strains were further grouped together in the resistant group for analysis. *E. coli* ATCC 25922 was used as the control strain.

Ethical considerations

The study received ethical clearance from the Ethical committee of the Obafemi Awolowo University Teaching Hospital, Ile-Ife, Nigeria.

Results

Over the study period, 162 randomly selected units of blood (160 refrigerated packed cells/whole blood and two platelet concentrates) were tested and 14 (8.8%) were found to be contaminated. All the contaminated samples were of whole blood. Table I shows the contaminated blood bags and the identified organisms.

All the bacterial species isolated were Gram positive, namely *Staphylococcus aureus*, coagulase-negative *Staphylococcus*, *Bacillus sp* and *Listeria sp* (Table I). The bacterial isolates showed diverse resistance patterns as shown in Table II. Resistance to all the antibiotics

Table I: Isolated microorganisms and time of storage

Microorganism	Time of storage
<i>Staphylococcus aureus</i>	Day 0
<i>Staphylococcus aureus</i>	Day 14
<i>Staphylococcus aureus</i>	Day 14
<i>Listeria</i> spp	Day 8
<i>Listeria</i> spp	Day 6
<i>Listeria</i> spp	Day 21
<i>Bacillus</i> spp	Day 1
<i>Bacillus</i> spp	Day 1
Coagulase-negative staphylococci	Day 1
<i>Bacillus</i> spp	Day 3
<i>Bacillus</i> spp	Day 0
<i>Staphylococcus aureus</i>	Day 12
Coagulase-negative staphylococci	Day 0
Coagulase-negative staphylococci	Day 5

Table II: Antibiotic resistance pattern of the bacteria isolated from contaminated samples : numbers = number resistant

Isolates	Number tested	AMP	COT	ERY	PEN	TET	RIF	CIP	GEN	CEF
<i>S. aureus</i>	4	NT	3	2	4	NT	0	4	1	0
CoNS	3	NT	3	3	3	NT	1	3	1	3
<i>Bacillus</i> spp	4	3	3	3	4	4	3	2	NT	NT
<i>Listeria</i> spp	3	3	3	3	3	3	3	3	NT	NT
Total	14	6/7	12/14	11/14	14/14	7/7	7/14	12/14	2/7	3/7

AMP = ampicillin; COT = cotrimoxazole; ERY = erythromycin; PEN = penicillin; TET = tetracycline;
RIF = rifampicin; CIP = ciprofloxacin; CEF = ceftriaxone; NT = not tested
CoNS = Coagulase-negative staphylococci

tested except gentamicin and ceftriaxone ranged from 50% to 100%.

No significant difference was found in the blood group of the blood in units contaminated by bacteria; blood group O positive (9/79; 11.4%), blood group B positive (3/33; 9.1%), blood group A positive (1/17; 5.9%) and blood group O negative (1/10; 10.0%) respectively.

The length of storage of the blood ranged from 0 to 21 days (mean = 4 days); most contaminated samples (57.1%) had <1 week of storage.

Discussion

The importance of the prevalence and source of bacterial contaminants of blood and blood components can not be over-emphasized particularly in the planning of preventive measures at blood transfusion centers across the world. In developed countries transfusion of blood and blood components has a low but known risk infection for patients, but remains a threat.^{10,11} Furthermore, the characterization of the bacterial isolates, types of blood or components contaminated and the antibiotic sensitivity pattern could be of public health importance and impact on clinical practice. The possibility and problem of bacterial contamination of blood and blood products has received very little attention in the African continent. Few countries in Africa; Ghana^{13,14} and perhaps Kenya have published records of bacterial contamination of blood or blood products.¹⁷ In the present study we report a prevalence of 8.8%. This rate is lower than that reported from Ghana, a neighboring West African country, but comparable to the 8.8% found in paediatric whole blood transfusion from Kenya.¹⁷ Strikingly, it is higher than the rates (0.2 and 0.15%) reported in the developed countries of United States¹⁸ and UK¹⁹ respectively.

The observed high rate in our study as well as the other reports from Africa underscores the need for further laboratory and clinical surveillance of blood transfusions to be undertaken in adults and children to clarify the extent and nature of the problem. It also raises concerns on the need for improved measures to ensure blood transfusion safety. Efforts should be targeted towards altruistic repeat blood donors as against the replacement or paid donors that are

common in our centers,²⁰ in addition to improved donor selection and screening, and adequate cleaning of phlebotomy sites.

The bacterial isolates from this study are similar to those reported in the literature,¹³⁻¹⁵ these organisms generally proliferate poorly during storage at 1 to 6°C. Being skin commensals, contamination is thought to occur primarily during phlebotomy, as a result of incomplete disinfection and/or skin core removal by the collection needle.²¹

Isolation of *Listeria* in blood products is unusual. Although widely distributed, it is rarely a commensal in humans. Interestingly enough all our three isolates occurred within a short time period. In 2004, the American Red Cross (ARC) of Southern California reported platelet products testing positive for *Listeria monocytogens*.²² About the same time, the Centre for Disease Control (CDC) found two other cases with the same pulse field gel electrophoretic patterns. Although the CDC report suggested that environmental contamination, false-positive laboratory results, and skin contamination are unlikely, we cannot completely exclude these factors in the present report as skin decontamination was done only with 70% alcohol. In line with the recent WHO guidelines for disinfection of phlebotomy sites, there is need to improve on skin disinfection for blood donors.²³

As previously noted by Adjei *et al*,¹⁴ we found that contamination was usually evident within a week of blood donation. Gram-positive skin commensals are isolated soon after donation but rarely from stored blood, where as psychrotropic (cold-tolerating) Gram-negative organisms are not usually detectable until after a period of proliferation during storage.²⁴

The high resistance rates in the organisms we isolated highlight the growing problem of antimicrobial resistance worldwide.²⁵ It also portends grave concerns should septicaemia develop post-transfusion and require antimicrobial therapy.

The study is limited by the inability to follow up the recipients of the blood units to determine clinical outcome of infection, furthermore we had very small units of blood components – platelets, plasma to

study. The rarity of other blood components is not unconnected with the small number of potential non-remunerated blood donors as well as scarcity of separating blood bags in the country.

Conclusion and recommendations

We conclude that bacterial contamination of transfused blood is common in our clinical practice. This portends a potential risk of hospital care associated infection to patients and with public health significance. The Nigerian National Blood Transfusion Policy introduced in 2006 should include protocols for prevention and control of donor blood contamination that can be adapted in transfusion centers across the country. Efforts have been made to ensure voluntary, unpaid donors, and we advocate the use of 70% alcohol with 2% chlorhexidine gluconate or tincture of iodine with for disinfecting phlebotomy sites, and of single-use towels for our blood bank units as recommended in the recent WHO guidelines.²³

Note

This work was presented in part (orally) during the 1st Infection Prevention Control African Network (IPCAN) congress in Kampala, Uganda 21-23, September, 2009.

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