INTERNATIONAL JOURNAL OF
SCIENCE ARTS AND COMMERCE

An Empirical Study on the Prevalence of Bacterial Contamination in Donated Blood in Dar Es Salaam City; Tanzania

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Abstract

Even though the proper laboratory techniques have been done to avoid blood reactions before transfusion, still there are blood reactions that have been reported and the source of these reactions are unknown .The purpose of this study was to investigate and analyze the prevalence of bacterial contamination in donated blood collected in Eastern zone blood transfusion centre within Dar es Salaam city. The two objectives guided the study: to determine the prevalence of bacterial contamination in donated blood and to describe the Antimicrobial sensitivity pattern of the isolated bacterial pathogens in the donated blood. This research was conducted using cross section survey with 384 respondents calculated using estimation method formulary : $N=Z^2P(1-P)/e^2$ Where by: N= Sample size Z=Standard normal deviate corresponding to two sided (1.96) P=Prevalence of bacteria pathogens in donated blood e= margin of error (5%) thus, $N=1.96^2$ X 0.5 (1-0.5)/ $(0.05)^2$ =384. A mixed method was adopted. The quantitative and qualitative data were acquired through observation checklist, documentary review and questionnaire methods as to gather information of respondents. The findings reveled that about 2.8% had bacterial contamination, of which 2.3% were gram positive cocci and 0.5% gram positive rods. Again, the bacterial isolates were about 63.6% coagulase negative staphylococci which were identified as staphylococcus epidermidis whereby 18.2% were micro cocci identified as

M.luteus and bacilli species identified as Corynebacterium diphtheroids. Again, sensitivity among the organisms varied; as all the 100% of the organisms isolated being sensitive to amikacin. According to these findings the isolates obtained in the donated blood are skin associated organisms and they are considered as contaminants related to procedure during donor bleeding.

Key words: *Prevalence, Bacterial Contamination, Donated Blood, Tanzania*

1. Introduction

Blood transfusion services are required to provide blood and components which are safe (Dodd et al, 2003) in cost effective way for transfusion into patients who require the blood products (Blachman, 2002). However, blood transfusion can be a potential source of infection by a variety of transmissible agents (Hillyer et al, 2003). Brecher et al, 2005 describes that human error occurs during the complex processing and this can be in the laboratory, collection, transportation of the blood and administration of blood to the patient, cause the blood to become contaminated with infectious agents. The screening process does not detect all the infectious agents in the blood as a result of low sensitivity of the tests (Armah et al, 2006) thus indicating failure to test for all infectious agents. In some cases, donors donate in the window phase of the infection when the numbers are too low for detection (Hillyer et al, 2003). Most often the bloods get contaminated with bacteria at the time of bleeding of donors (Dodd et al, 2003). The storage period may serve as incubation period for the low numbers to proliferate before it is transfused with the resultant reduced blood pressure, shock and collapse (Engelfriet et al, 2000).

Moreover, Transfusion related transmission of Human Immunodeficiency Virus, Hepatitis B and Treponema have steadily decreased due to the rigorously screening efforts by transfusion services, but the risk of transmission of other bacteria and malaria has remained high (Wagner et al, 1994). Bacteria which commonly contaminate blood are able to multiply in refrigerated blood to high concentrations to initiate infection in the transfused patient especially blood that is stored for a long time in excess of 32 days (Bladley et al, 1997). Again, medical literature during the past years is replete with case studies of apparent sepsis predominantly due to bacteria from normal skin flora (Goldman et al, 1991). The commonly reported skin microorganisms include Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus species and Corynebacterium species (as common blood contaminants). Bacillus species and Gram negative organisms such as Yersinia enterococolitica, Pseudomonas sputida, Escherichia coli, Enterobacter aero benes, atialiquifaciens, Campylobacter jejuni, Enterobacter species, and Salmonnella species are other bacterial isolates reported from donor blood (Morel et al, 2003). Immunosuppressed patients and older individuals with poor nutritional status are most susceptible population, but healthy individuals can have a rapidly fatal outcome when transfused with a large load of bacteria alone or with endotoxins (Wong, 2004). In resource-limited settings, blood is collected most commonly in whole blood units. The World Health Organization (WHO) estimates that

resource-limited countries should begin to fulfill baseline clinical demand if 10-20 whole blood units per 1,000 populations are collected each year (Tapko et al, 2007). To improve blood supply adequacy and transfusion safety, WHO has recommended that resource-limited countries adopt comprehensive national policies for national blood transfusion services (WHO, 2002).

2. Research Problem

The success with screening viral pathogens, bacterial contamination now has the dubious distinction of being the most common infectious risk from transfusion and has became a matter of increasing concern and attention (Morel et al, 2003). Even though the proper laboratory techniques have been done to avoid blood reactions before transfusion, still there are blood reactions that have been reported and the source of these reactions are unknown (Breacher et al, 2003). In contrast, bacterial contamination has been shown to be the cause of transfusion reactions in some cases though the blood was properly cross matched (Hillyer et al, 2003). In the study conducted at Tamale Teaching Hospital Ghana one of African country the prevalence shows that 17.5% grew isolates of various bacteria from donated blood (Opoku-Okrah et al, 2009). If there will be no any intervention to this problem, the risk of transfusing bacterial pathogens to the recipients/patients will still continuing and cause problem to the society. That is why this study is imperative to be conducted.

3. Literature Review

As far as foreign studies are concerned, globally, the study on blood donation by WHO in sub-Saharan countries found that approximately 80 million units of blood are donated each year (WHO, 2002). Of this, a total of 2 million units (2.5%) are donated in sub-Saharan Africa, where the need for blood transfusions is greater because of maternal morbidity, malnutrition, anaemia from various causes and a heavy burden of infectious diseases such as malaria (WHO, 2002). In 2004, blood collections in most of the 14 U.S. President's Emergency Plan for AIDS Relief (PEPFAR) supported countries did not satisfy clinical demand. Inadequacy of the blood supply in many African countries was compounded by inconsistent laboratory screening for HIV infection and collection of blood from donors at greater risk for HIV infection (WHO, 2002). Collections often were coordinated by hospital-based services that frequently relied on paid donors or replacement donors (e.g, family members of patients) who typically were at greater risk for HIV infection and, because of external pressures to donate, might not have revealed their behavioral risks for HIV during donors selection (McFarland et al, 1997). HIV screening of donor blood in non standardized laboratories without quality assurance further increased the risk for transfusion-associated HIV transmission (McFarland et al, 1997).

Each year, approximately 13,898,000 units of red blood cells or whole blood are transfused in the United States alone (Bethesda, 2003). This equates to one unit being transfused every 2.3 seconds. Despite this large number, sepsis associated with the transfusion of bacterially contaminated red blood cells components is generally regarded as a very rare event (McDonald et al, 2005). From 1976 through September 1998, 26 fatalities thought to be secondary to contaminated whole blood or red cells were reported to the U.S Food and Drug

Administration (FDA) (Jafari et al, 2002), approximately one red cell-related death per year has been reported. The majority of deaths reported to the FDA involved Yersinia enterocolitica. The highest reported incidence of Y. enterocolitica contamination was reported in New Zealand, with an incidence rate of 1 in 65,000 and a fatality rate of 1 in 104,000 red cell units transfused (Sen, 2000). Recent passive reporting studies of bacterially contaminated red cells from the United States, France, and the United Kingdom and Canada that caused symptoms of infection show a relative small number of Yersinia cases (Hoppe, 1992) in France and United Kingdom. Of the reported deaths, one was due to a coagulate-negative Staphylococcus (Hoppe, 1992). Bacterial contamination of transfusion products, especially platelet, is a longstanding problem that has been partially controlled through modern phlebotomy practices, refrigeration of red cells, freezing of plasma, and improved materials for transfusion product collection and storage. Indeed, bacterial contamination of platelet products has been acknowledged as the most frequent infectious risk from transfusion occurring in approximately 1 of 2,000–3,000 whole-blood derived, random donor platelets (hereafter RDP), and apheresis-derived, single donor platelets (Jacobs et al, 2001). Where as in Canada, the estimated residual risk of contamination of blood products with bacterial agents is 1 in 5,000 for platelets and 1 in 30,000 for red blood cells (Kleinman, 2006). It has been proposed that the higher incidence of bacterial transmission via platelets is due to the difference in the storage temperatures; also important is the duration of storage, which has a direct correlation with the likelihood of bacterial contamination (Yomtovian et al, 1993). Bacterial contamination in USA is considered the second most common cause of death from transfusion (after clerical errors) with mortality rates for platelet-related sepsis ranging from 1:20,000 to 1:85,000 donor exposures (Ness et al, 2001). Estimates of severe morbidity and mortality range from 100 to 150 were transfused to individuals each year (Yomtovian et al, 1993). From 1976 through September 1998, 51 fatalities thought to be secondary to contaminated platelets were reported to the U.S. FDA (Jafari et al, 2002), gram-negative organisms accounted for the majority of deaths (59.7%). Similarly, passive surveillance studies from the United Kingdom, the United States, and France show that gram-positive organisms were implicated in 41 (71%) of 58 of cases but gram-negative organisms (mostly members of the Enterobacteriaceae) account for the majority (82%) of 11) of the fatalities (Rudi et al, 2004).

In Africa, several infectious diseases have been found to be associated with transfusion of blood and blood components. *In Cameroon; it was* found significant *Salmonella* antibody titers in more than 10% of apparently donated blood (Nsutebu *et al, 2002). H*owever in Nigeria, a study was conducted out of the 200 specimen which were analyzed, 106 (53%) were found to be Widal-positive , *S. typhi* (D) was the commonest bacteria which shown high titers in reaction (48.6%) in donated blood (Teddy et al, 2009). In case of East Africa, Bacterial contamination of pediatric whole blood transfusions in Kenyan hospital showed that 44 bacterial contaminants were isolated from 38 blood packs-an overall contamination frequency of 8.8% (95% confidence interval, 6.1%-11.4%). Sixty-four percent of the bacteria isolated were Gram-negative. Many of the isolates are usually found in the environment and the most likely source of contamination was considered to be the hospital blood bank (Hassall et al, 1993). Bacterial contamination of whole blood may be a significant but unrecognized

hazard of blood transfusion for children in sub-Saharan Africa (Hassall et al, 1993). The studies which were conducted from the period 1980–2009 indicated that the median prevalence of malaria among 33,029 blood donors was 10.2% (range, 0.7% in Kenya) (Alex et al, 2010).

With Local studies in Tanzania, the demand for blood transfusion services is high due to endemicity of infections causing anemia, malnutrition, surgical and obstetrical emergencies associated with blood loss (Gumodoka, 1993). There is an increase of voluntary donors from 20% to 80% in 2006 t0 2009 respectively and an increase in blood collection from 52,000 units in 2006 to 114,000 units in 2008 (WHO, 2009). A major factor contributing to the demand for blood transfusion is its use as the last resort treatment for anaemia, which is highly prevalent in the country. Due to increase of blood demand in Tanzania, the donated blood must be further undergoing scientific laboratory investigation to identify proliferated bacteria.

4. Methodology

A cross-sectional study was conducted at Eastern Zone Blood Transfusion Services located at Mchikichini, Ilala District, in Dar es Salaam. This cross-sectional study conducted between December 2010 and January 2011. Stored whole blood bags were selected from the refrigerator containing 500 blood bags using a simple random sampling technique. About 384 blood bags were randomly picked for study, each blood bag was given an ID number (1-500), and a table of random numbers was used to select the 384 donated blood bags. This sample size was calculated using the following formula:

$N=Z^2P\ (1-P)/e^2$

Where by:

N= Sample size

Z=Standard normal deviate corresponding to two sided (1.96)

P=Prevalence of bacteria pathogens in donated blood

e= margin of error (5%)

$N=1.96^2$ X 0.5 (1-0.5)/ $(0.05)^2$

N=384,

It is therefore the sample size is 384.Moreover, culture was done on different media; isolates were identified using standard biochemical and bacteriological methods. Kirby- Bauer disk diffusion method was used for antimicrobial susceptibility testing according to existing guidelines of CLSI. The Questionnaires/donors' records and Check lists, were used to record each blood unit daily taken from blood bank.

4.1 Laboratory Methods

4.1.1. Blood Culture

The cultured blood bottles were observed daily for possible signs of bacterial growth (pellicle formation, hemolysis, turbidity). After incubation a loopful of each broth suspension was sub cultured onto Sheep blood agar, chocolate agar and MacConkey agar plates every day in three consecutive days. The Sheep blood agar and MacConkey agar plates were incubate aerobically and Chocolate agar was incubated at 5-10% CO_2 for (18-24 hours). After overnight incubation the plates were inspected for bacteria growth. The bacterial growths were identified by their colonial morphology, grams reactions, and biochemical test and sugar test (lactose and non lactose fermentation).

4.1.2 Colonial Morphology

Staphylococcus species: On a sheep blood agar the Staphylococcus species appeared as white, non-pigmented colonies without hemolytic after incubation which measures (2.5-6 mm).These were identified as Staphylococcus epidermidis.

Micrococcus species: The colonies on sheep blood or chocolate agar the plates were observed macroscopically which appeared as small, convex, non-hemolytic, variably pigmented yellow colonies and identified as M.luteus.

Bacillus species: The colonies on the Sheep blood agar were observed macroscopically which appeared as large (2 - 7 mm) with a frosted-glass appearance, but may become opaque.

4.1.3 Gram Stain

The colonies were tested by their gram reactions to categorize into two groups; gram positive or gram negative bacteria, or whether cocci or rods.A drop of normal saline was placed on a clean glass slide. Using sterile wire loop (by flaming) a colony of the culture was emulsified on the sterile saline to form a thin film and this was heat fixed and Gram staining was performed; The film was placed on a staining rack over a sink and it was covered with 0.5% gentian violet for 1 minute, then it was washed with a thin stream of clean water to remove the excess stain. Then the film was covered with Lugol's iodine and left for 1 minute, the smear was washed in a thin stream of clean water and also decolorized with 50% acetone alcohol solution slowly, one drop at a time and stop as soon as no more blue colour comes out of the smear, then it was counterstained with dilute carbol fuchsine for 30 seconds. The smear was washed in a thin stream of clean water to remove excess stain and it was dried. A drop of immersion oil was added on the smear, and was placed on the microscope stage. This was examined under oil immersion objective (100 X magnifications) for the presence of Gram positive or Gram negative bacteria.

5. Analysis and Results

5.1. Objective One: Bacterial Isolates Among Donated Blood

Of the 384 donated blood bags from refrigerator ,the donors records show that, those who were not feeling well and health 10 (2.6%), received any treatment 1 (0.3%), had any operations, injections and vaccinations 4 (1%), had typhoid 0 (0.0%), any long term illness like epilepsy were 11(2.9%), had a stab would or needle stick injury 6 (1.6%), injected yourself or been injected, besides in health facility 1 (0.3%), and those who considered their blood safe 4 (1%) as shown in Table 1.

Table 1: Clinical History of the Individuals Donated Blood

Variable	Frequency	(%)
Not feeling well and good health	10	(2.6)
Been ill, received any treatment	1	(0.3)
Had any operations, injections and vaccinations	4	(1.0)
Had typhoid	0	(0.0)
Any long term illness like epilepsy	11	(2.9)
Been injected, besides in health facility	1	(0.3)
Do you consider your blood safe?	4	(1.0)

Source: Field Data

5.2 Objective Two: Sensitivity Testing

Amikacin was shown to be 100% sensitive to these organisms as seen in table 2 below. Of which 10 (100%) Staphylococcus epidermidis were sensitive to ciprofloxacin, gentamycin, cefriaxone, erythromycin and co-trimoxazole. Gram positive rods were tested against erythromycin and Gentamycine; where 100% were sensitive to Gentamycine and (60.5%) were sensitive to erythromycin. Micrococcus species were tested against ceftriaxone and gentamycin had (98%) and (97.5%) sensitivity to these antibiotics respectively. Gram positive rods showed resistant to ampicilin, contrimoxazole and Tetracycline which is 100% resistant to these antibiotics. The results are shown in Table 1:

Table 1 : The Sensitivity Pattern of the Various Organisms Isolated

Antimicrobial Agent	Staphylococcus epidermidis	Gram positive rods (Corynebacterium diphtheroids)	Micrococcus species(M. luteus)

Ampicilin	49.5	0	35
Amikacin	100	100	100
Ciprofloxacin	100	99.2	75
Cotrimoxazole	100	0	14.5
Ceftriaxone	100	82	98
Erythromycin	100	60.5	93.6
Gentamycin	100	100	97.5
Penicillin	55.5	67	100
Methicilin	0.5	45	75
Tetracycline	100	0	34

Source: Field Data

6. Discussion

In light of the findings within view of the results obtained in this and in past studies on this topic. The findings reveled that there was low bacterial contamination in this centre, which was about (2.8%) and most of them were non pathogenic bacteria. This finding is considered low compared to reports from other studies. We again found similar works reported low levels in Ghana but other scientists have reported levels as high as 17.5 % (Opoku et al, 2009), (Mac Donald et al, 1998) and (Morel et al, 2003) which are higher than values obtained in this study as to compare with past studies follows.

This study revealed the presence of staphylococcus epidermidis, micrococci,and gram positive rods which alike to the work conducted by other studies which categorized the blood units into Group I to VIII showed that a of total 214 units in Group VI, only three (1.4%) showed evidence of bacterial contamination, with two occurrences of coagulase-negative Staphylococcus species plus an unclassified gram-negative rod and one instance of coagulase-negative Staphylococcus species plus Pseudomonas paucimobilis (the latter was considered a contaminant of the inoculation process during culture set-up). In total, of 523 RDP units cultured (309 from Group V and 214 from Group VI), 2.9% (assuming 12 units contaminated in Group V) showed evidence of bacterial contamination. A total of 43 units in Group VII, only two (4.7%) showed evidence of bacterial contamination, where one unit grew gram-variable rods, and the other grew an Enterococcus species. About 54 units in Group VIII none was positive for bacterial growth or signs of contamination. As a whole, of 97 units cultured from 13,641 units produced, two units (2.1%) showed evidence of

contamination. (Perez et al, 2001). Although the mean prevalence of bacterial contamination in whole blood derived, RDP platelets is 33.9 per 100,000 units and for apheresis-derived, platelet units is 51.0 per 100,000 units. For RBCs it is 2.6 per 100,000 units (Hillyer, et al, 2003).

The Eastern Zone Blood Transfusion Services Centre itself has no data on blood bank bacteraemia, transfusion related sepses or mortality records for comparison, as reported by others (Perez et al, 2001), (Ness et al, 2001) because it is perceived that transfusion related sepsis or mortality are rare phenomena. We linked these findings to those by Brecher et al, 2004 and found that probably low quantity of inoculums of bacteria occurs in the blood which only results in transient bacteraemia as a result of antibiotic coverage which masks the signs and symptoms. Proper blood donor skin disinfection has long been recognized as the definite way to reduce blood contamination. (Brecher et al, 2005).

Again, the findings within view of the results indicated that the cultures performed on the whole blood were considered with the aim of studying two aspects of contamination of blood components: the presence of transient asymptomatic episodes of bacteraemia in blood donors and the presence of a higher bacterial load (due to collection of contaminated skin with the needle) in the first milliliter of collected blood. Practically, these aspects were of only slight relevance. The cultures performed on the blood showed a prevalence of contamination of 2.8%, and the only positive sample was contaminated by a common skin contaminant (Staphylococcus epidermidis). Culture method performed using Bact/ALERT aerobic bottles was not able to prevent transfusion of the only contaminated blood identified in this study however according to past studies in the recently we have found reported that false negative results that are also possible with culture methods (Larsen et al, 2005). Other screening methods proposed to reduce the risk of sepsis due to contaminated platelets have shown lack of sensitivity or specificity (Yazer et al, 2005) (Schmidt et al, 2005) or are too expensive (Mohammadi et al, 2005). We therefore, suggest that, in compliance with regulatory agencies, pathogen inactivation systems suitable for cellular components should be a more effective approach to reducing the risk of transfusion-associated sepsis than an approach based upon the screening tests currently available (blajchman et al,2005).

Over again, the isolates obtained in the study were mostly skin associated organisms and are often considered contaminants related to either procedure during donor bleeding (Morrow et al, 1991), or of taking the sample for culture (Alvarez et al, 1995). Bacterial contamination caused by coagulase negative staphylococci, including other skin microbes like the diphtheroids (gram positive rods) are difficult to demonstrate as ‘true pathogen’, and therefore can be ignored in routine diagnosis. Bacteraemia caused by such bacteria can lead to deleterious consequences for the immunocompromised patients (Perez et al, 2001) such as the premature and newborns. Also the presence of M. luteus indicates contamination; these organisms are found in many places such as the human skin, water, dust, and soil. Micrococcus is generally thought of as harmless bacterium, but there have been rare cases of Micrococcus infections in people with compromised immune systems, as occurs with HIV patients (Smith et al, 1999). Gram positive rods the Corynebacterium diphtheroids were

resistance to ampicilin, contrimoxazole and tetracycline antibiotics similarly to previous studies about antimicrobial resistance. According to Okeke et al, 2007, Adjei, 2004 , Milles –Robertson et al , 2003 the risks of transfusing bacterially contaminated donor blood is high and transfusing blood with multidrug resistant strains of bacteria may worsen the plight of the already sick and the immunocompromised. Also the resistance of Gram positive rods to Ampicilin, Tetracycline and contrimoxazole which were almost similar to the study done by (Adjei et al, 2009) whereby all the isolated gram positive organisms were resistant to Cefuroxime, penicillin,ampicillin, and Cotrimoxazole but sensitive Cloxacilin, Tetrecyclin, Erythromycin and Gentamycin. Similarly, all the Gram negative organisms isolated were obser5ved in this study that resistant to Cotrimoxazole except Y.enterocolitica Tetracyclin Ampicilin, Cefuroxime, Cotrimoxazole and Chloramphenical exists. That is the Bacterial contamination in donated blood is very low at the Eastern Zone Blood Transfusion Services Centre. Having compared the results and past studies we have discovered that good automated machines may minimizes the chance of bacteria in blood donated.

7. Conclusion

The study findings conducted at Mchikichini in Ilala District in Dar es Salaam region confirms the blood contamination among donated blood is not high, but still there is contamination of bacteria agents, this highlights proper follow up of all procedures during blood donation processes. Also the isolates obtained in the donated blood are skin associated organisms and are considered as contaminants related to procedure during donor bleeding. The study concludes that there is a need to improve existing facilities at the blood storage centers. This can in turn reduce on the risks of contamination of blood units.

8. Recommendations

There should be improvement on prevention control measures by improving on the existing facilities at the blood storage centers to reduce on the risks of contamination of blood centers in Tanzania. Again, Blood collectors and quality assurance staffs should adhere to safety precautions, protocols/quality assurance especially during blood collection and any other procedures involving blood so as to reduce the risk of contaminating blood units.

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