THE PREVALENCE OF BACTERIAL CONTAMINATION IN DONATED BLOOD IN DAR ES SALAAM, TANZANIA, 2010.

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THE PREVALENCE OF BACTERIAL CONTAMINATION IN DONATED BLOOD IN DAR ES SALAAM, TANZANIA, 2010.

By

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A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Epidemiology and Laboratory Management of the Muhimbili University of Health and Allied Sciences

Muhimbili University of Health and Allied Sciences

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for examination of thesis/dissertation entitled, ***the prevalence of bacterial contamination in donated blood in Dar es Salaam, Tanzania 2010,*** in fulfillment of the requirements for the degree of Masters of Applied Epidemiology at Muhimbili University of Health and Allied Sciences.

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DECLARATION AND COPYRIGHT

I, **Malimu, Emmanuel Zephania (**Candidate MSc. Applied Epidemiology and Laboratory Management), declare that this dissertation is my own original work and that it has not been presented and will not be presented to any other university for a similar or any other degree award.

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Dedication

This work is dedicated to my Parents Mr. Zephania Malimu (The late), my beloved Mother Mrs. Sophia Malimu for all the moral support which was accorded to me throughout the period of my studies.

Abstract

Introduction: Blood transfusion services are required to provide blood and components which are safe in cost effective way for transfusion into patients who require the blood products.

Study objective: This study aims to determine the prevalence of bacterial contamination and the antimicrobial resistance pattern in collected blood in Eastern zone blood transfusion centre.

Methodology: A cross-sectional study was conducted at Eastern Zone Blood Transfusion Services located at Mchikichini, Ilala District, in Dar es Salaam. 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. 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. Data entry and analysis were performed using EpiInfo 3.5.1.

Results: About 11 (2.8%) were found to have bacterial contamination, of which 9 (2.3%) were gram positive cocci and 2 (0.5%) gram positive rods**.** The bacterial isolates were about 7 (63.6%) coagulase negative staphylococci identified as *Staphylococcus epidermidis* and 2 (18.2%) were micro cocci identified as *M.luteus* and bacilli species identified as *Corynebacterium diphtheroids.* Sensitivity among the organisms were varied; as all the 11 (100%) of the organisms isolated were sensitive to amikacin, of which 7 (100%) *Staphylococcus epidermidis* were sensitive to ciprofloxacin, gentamycine, 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. *M.luteus* were tested against ceftriaxone and gentamycin had (98%) and (97.5%) sensitivity to these antibiotics respectively. Gram positive rods showed (100%) resistant to ampicilin, cotrimoxazole and tetracycline

Conclussion: The isolates obtained in the donated blood are skin associated organisms and are considered as contaminants related to procedure during donor bleeding or taking sample for culture.

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Acronyms

AFENET	Africa Field Epidemiology Network
ATCC	American Type Culture Collection
BA	Blood Agar
CA	Chocolate Agar
CI	Confidence interval
CLSI	Clinical Laboratory Standard
DNAse	Deoxyribonuclease
EZBTS	East Zone Blood Transfusion Centre
FDA	Food and Drug Administration
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
MCA	Mc Conkey Agar
MoH&SW	Ministry of Health and Social Welfare
SPHSS	School of Public Health and Social Sciences
TFELTP	Tanzania Field Epidemiology and Laboratory Training Program
WHO	World Health Organization

1. Introduction

1.1. Background:

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 stated 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).

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).
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).

1.2. Literature review

Globally, 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 that caused symptoms of infection show a relative small number of *Yersinia* cases (Hoppe, 1992). 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)

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). However 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 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).

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.

1.3 Public Health Importance of Bacterial pathogens in Donated blood:

The most common infective agents to be transmitted through blood transfusion are, in fact, bacteria (Mudassar *et al*, 2008). The testimony to this fact is extensive data from studies like the French Haemovigilance Bacthem study (regarding determinants of transfusion-associated bacterial contamination) (Perez *et al*, 2001), the US BaCon study (regarding transfusion-transmitted bacterial contamination in the US from 1998 through 2000) (Williamson *et al*,1999) and the United Kingdom Serious Hazard of Transfusion (SHOT) program (data collection in the UK and Ireland regarding deaths or major complications of transfusion of blood or its components) (Williamson *et al*, 1999). The incidence of bacterial transmission depends on the blood product and also on the definition of the cases (Mudassar, 2008).

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).

1.4. Problem statement

Bacterial Contamination of Blood is rare but a very serious complication of blood transfusion. Currently, in Tanzania, the established zonal blood banks supply do not screen for bacterial contamination and this pose a risk for transmission of pathogenic bacteria.

Thus due to 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.

1.5. Rationale

No study has been conducted in Tanzania to establish whether or not bacterial contamination occurs in stored donor blood before transfusion, the extent of bacterial contamination of blood donated for transfusion is not known. Transfusion of bacterially contaminated blood can result in sepsis and will constitute a substantial health burden to the patient. The potential dangers and consequences of transfusing multi drug resistance bacteria are rather substantial.

An estimated 80 million units of blood are donated each year (WHO, 2002), about 2-million units 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). Blood transfusion is coordinated by hospital-based services that frequently rely 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. Tanzania is one among Sub-Sahara African countries which is in one way another shares common health problem and health challenges among other Sub-sahara African countries, it is therefore; this study will generate valuable findings on the magnitude of bacterial contamination and the antimicrobial resistance pattern in collected blood in Tanzania.

2.0. Objectives

2.1. General objective

To determine the prevalence of bacterial contamination and the antimicrobial resistance pattern in collected blood in Eastern zone blood transfusion centre.

2.2. Specific objectives

2.2.1 To determine the Prevalence of Bacterial contamination in donated blood

2.2.2 To describe the Antimicrobial sensitivity pattern of the isolated bacterial pathogens in the donated blood.

2.3. Hypotheses/research questions

2.3.1 What is the magnitude of bacterial contaminated among donated blood in Tanzania?

2.3.2 What is the magnitude of the risk factors for bacterial contamination among donated blood?

2.3.3 Is there an association between identified risk factors and among donated blood?

3.0 Methodology

3.1. Study area

This study was carried out at the Eastern zone blood transfusion centre which is located at Mchikichini in Ilala District in Dar es Salaam region. It is one of the eight zones of blood transfusion centers in Tanzania. It serves four regions which are Dar es Salaam, Coast, Morogoro and Dodoma and 68 hospitals.

3.2. Study design

This was a cross-sectional study conducted between December 2010 and January 2011.

3.3. Donated blood selection criteria;

All of the following donated blood bags were included in the study;

- Donated blood bags of donors aged between 18- 65 years old.
- Donated blood bags of donors weighing > 50 kilogram.

Note; These information were obtained from donors stored records (questionnaire).

3.4. Sample size estimation

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

3.5. Data, Sample collection and Testing

The following tools were used to collect data in this study;

Questionnaires/donors' records included demographic information like sex, age, marital status, weight, Hemoglobin level and the health status of the donor

Check lists, which was used to record each blood unit daily taken from blood bank

3.5.1. Laboratory Sample collection

Blood samples were collected daily according to the availability of blood units in the blood bank.

3.5.2. Blood collection procedure

Simple random selection technique was used to select 384 blood bags from the refrigerator containing maximum of 500 blood bags where by the Blood bags were tagged using a specific ID number 1-500 on a slip of paper and lottery was conducted, then the selected numbers of particular blood unit was matched with the donor's records with the registration number and demographic information which are present on the questionnaire, (this questionnaire is provided by the zonal blood bank contains demographic and clinical information of the donor.

The blood bag was sterilized by methylated spirit then was brought at the bench which was sterilized with 10% Sodium hypochlorite. The cords were folded into four parts the quarter closest to the bag was chosen as a site for puncture. Electrical Hot sealer and sterilized forceps were used, and they were also sterilized before and after drawing the blood. Before delivering the blood into the culture bottle (Bact/ALERT SN –Aerobic) broth (Biomerieux inc.Durham, NC 27704) bottle was sterilized by using methylated spirit (alcohol 70% v/v).

The total volume of 5mls of blood was drawn and delivered into the bottle of 40mls capacity. Then the cap of the bottle was sterilized with methylated spirit and was packed into the cold box. The bottles were incubated at 37 degree Celsius.

3.6. Laboratory Methods

3.6.1. 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 as explained below:-

3.6.2. Identification of bacteria

The bacterial growths were identified by their colonial morphology, grams reactions, and biochemical test and sugar test (lactose and non lactose fermentation).

3.6.2.1 Colonial Morphology

Staphylococcus species: On a sheep blood agar the *Staphylococcus species* appeared as white, non-pigmented colonies without hemolysis 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.

3.6.2.2. 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 fuchsin 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.

Staphylococcus species:

These microscopically appeared as gram-positive cocci 0.5-1.5 μm in diameter occurring singly, in pairs or short chains, and as irregular grape-like clusters.

Micrococcus species:

Microscopically were appeared as Gram-positive cocci 1-1.8 μm in diameter forming pairs, tetrads (predominantly), or irregular clusters.

Bacilli species:

In the gram reaction under microscope appeared as gram positive rods, pleomorphic, slightly curved with tapered or clubbed ends. Cells may occur singly or in pairs, often in a

"V" formation (forming "Chinese letters"). Cells were stained weakly and unevenly giving a beaded appearance.

3.6.2.3. Biochemical test(s)

Bacteria colonies of *Staphylococcus, Micrococcus and Bacilli species* were identified by using Catalase, oxidase, Coagulase and Novobiocin tests.

Staphylococcus species

Catalase Test was used to identify these species; By using sterile glass rod a good growth of the test organism was removed from the media and immersed in the test tube containing 2-3mls of hydrogen peroxide solution. Immediate bubbling was observed which indicate the presence *Staphylococcus species* because they do produce the enzyme catalase which breakdown the hydrogen peroxide into water and oxygen. This distinguishes them from *Streptococci.*

Coagulase test: A colony of growth was taken from the media and mixed with plasma after 2-3 min coagulation was observed. Coagulase test was used to differentiate Staphylococcus aureus which produces the enzyme coagulase, from *Staphylococcus epidermidis* and Staphylococcus *saprophyticus* which do not produce coagulase.

Novobiocin Test;

By using Kirby-Bauer test technique to inoculate each Mueller-Hinton plate with one of the three *Staphylococcus* species; A diluted stock culture was prepared by transferring 0.25 ml of stock culture into a labeled, sterile tube containing 5 ml . mixed well by finger vortexing, a sterile swab was dipped into the diluted culture, the excess inoculum was removed by pressing the saturated swab against the inner surface of the tube, the swab was used to streak the surface of the plate in a horizontal direction, then the plate was turned at 90° and the surface was streaked in the other direction.

Then when the surface of the plate appears dry, a Novobiocin disc was placed at the centre of the Mueller-Hinton plate. And the plates was Incubated for 48 hours. After incubation, the zone size was measured. Any measurement equal to or less than 16 mm indicates resistance to Novobiocin. A zone 17 mm or larger indicates susceptibility to Novobiocin.

NB.Novobiocin test was used to identify *Staphylococcus epidermidis* from *Staphylococcus saprophyticus.*Therefore the test identified *Staphylococcus epidermidis.*

Micrococcus species

The catalase Test was used to identify the Micrococcus species: A colony from the media was picked by using a sterile glass rod and immersed into the 2-3mls of hydrogen peroxide in the test tube. Immediately the bubbles appeared this indicated the presence of Micrococcus species.

Oxidase test was used to identify the species of Micrococci: a colony from media was picked by using sterile glass rod and The impregnated disk (NNNN'-tetra methyl-p-phenylenediamine (TMPD) was wet with about 4 inoculating loops of de-ionized water ,by using a loop to aseptically transfer a large mass of pure colony of micrococcus to the disk. Then the disk Observed up to 3 minutes. The area of inoculation turned to dark blue to maroon almost black, then the result was positive and the organism was identified as *M.luteus.*

Bacillus species:

Catalase Test: These inoculums were taken from the media growth and a colony from the media was picked by using a sterile glass rod and immersed into the 2-3mls of hydrogen peroxide in the test tube. Immediately the bubbles appeared this indicated the presence of *Corynebacterium dipthroids*.

3.7. Antibiotic Susceptibility Testing

The susceptibility of isolates to selected antimicrobial agents was determined by the disc diffusion method and the Kirby Bauer method using antibiotics-impregnated paper discs (CLSI, 2000) containing the following Antibiotics with concentrations as shown below were used: Ampicillin 10mg, Gentamycin 10µg, Cotrimoxazole 25µg, Cefuroxime30µg, Penicillin 1.5IU, Tetracycline 30µg, Erythromycin5µg, Cloxacillin 1.5IU Ceftriaxone 30µg and Chloramphenicol 30µg. Briefly a sterile straight wire with a pure colony of the test isolate was inoculated into peptone water and the turbidity was adjusted to the equivalence of 0.5 McFarland opacity standards. A sterile swab was used to seed colonies into Muller-Hinton Agar to give a confluent bacterial growth. Using a pair of sterile forceps the antimicrobial discs were placed on the dry agar surface as appropriate. Then the plates

were incubated aerobically at 37ºC and read after 18-24 hours of incubation. Zones of inhibition surrounding the discs were measured using a vernier caliper.

The organisms were reported as "Sensitive" intermediate, or "Resistant" as described by CLSI. Quality control strains were included in the testing and these were *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa*, TCC 27853 and *Staphylococcus aureus* ATCC 29213.The criteria of selecting these antibiotics was by considering the pathogenicity and sites of infection of particular organism (Cheesbrough, 2006)

3.8.0. Quality assurance

This ensured that the information generated from the laboratory is accurate, credible and reproducible. This was achieved by assessing the quality of specimen, source of culture media, preparation of culture media, culture procedures, biochemical tests, and staining procedures. Dehydrated or commercially available media was used and prepared according to the directions of the manufacturers. The pH of each media was checked using pH meter. For sterility check 3 plates of the batch were incubated at 37^0 C for 48 hrs and the ability to support growth of the suspected organisms were determined by inoculating the medium with a typical stock culture.

Negative and positive controls were used to determine the biochemical response of the reagents/test kits. Test to detect intestinal parasites and enteric viruses were performed according to manufacturers' instruction.

3.9.0. Data analysis

Data were analyzed using SPSS 17.0 for Windows NT (SPSS, Inc, Chicago, IL) and Epi info. The χ^2 tests and Fisher's exact test were appropriate (College Station, TX) was used for statistical comparisons of categorical data.

3.10.0 Ethical consideration

Ethical clearance to conduct this study was obtained from the Higher Degree Research and Publication Committee of Muhimbili University of Health and Allied Health Sciences, Dar

es Salaam Tanzania. The permission to conduct the study was obtained from EZBTS Authority.

4.0. Results

4.1. Prevalence of bacterial contamination in Blood samples

A total of 384 samples from donated blood were included in the study, 11 (2.8%) found to have bacterial growth, of which 9 (2.3%) gram positive cocci and 2 (0.5%) gram positive rods.

4.2. Bacterial isolates among donated blood

The bacterial isolates among donated blood, were about 7 (63.6 %) coagulase negative staphylococci identified as *Staphylococcus epidermidis* and 2 (18.2%) were Micrococcus species, identified as *Micrococcus luteus* and *bacilli species* identified as *Corynebacterium diphtheroids.*

4.3. Sensitivity testing

Amikacin was shown to be 100% sensitive to these organisms as seen in table 1 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.

4.4. Clinical characteristics of blood donors

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 2.

Table 1 : The percentage 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

Table 2: 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)
Had a stab would or needle stick injury	6	(1.6)
Injected yourself or been injected, besides in health facility	1	(0.3)
Do you consider your blood safe?	4	(1.0)

5.0. Discussion

The aim of this study was to assess the prevalence of bacterial pathogens in donated blood in Dar es Salaam, we noted 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. 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.

This study revealed the presence of *Staphylococcus epidermidis*, micrococci,and gram positive rods which enveil similarly 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. (Enrique, Alvarez and Benjamin Lichtiger, 1994). 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. Probably low quantity of inoculums of bacteria occurs in the blood which only results in transient bacteraemia on transfusion (Goldman *et al*, 1991) or as a result of antibiotic coverage (Brecher *et al*, 2004) 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).

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 it was recently reported that false negative results 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). It has, therefore, been suggested 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).

The isolates obtained in this 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). Bacteria 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 (Okeke *et al*, 2007, Adjei, 2004 , Milles –Robertson *et al* , 2003) and elsewhere (Ozumba *et al*, 2005)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.

Further more the resistace of Gram positive rods to Ampicilin,Tetracycline and contrimoxazole which were almost similar to the study done by (Adjei *et al*, 2009) where by all the isolated gram positive organisms were resistant to Cefuroxime, penicillin,ampicillin, and Cotrimoxazole but sensitive Cloxacilin, Tetrecyclin, Erythromycin and Gentamycin. Similary, all the Gram negative organisms isolated were resistant to Cotrimoxazole except *Y.enterocolitica* Tetracyclin Ampicilin, Cefuroxime, Cotrimoxazole and Chloramphenical.

The Bacterial contamination in donated blood is very low at the Eastern Zone Blood Transfusion Services Centre; they do have the a sensitive questionnaire which captures the good donors who qualifies to donate blood, good automated machines which minimizes the chance of bacteria.

6.0. Limitation (s)

Inability to make a follow up for the recipients whether will develop any kind of septicemia as result of transfusion of contaminated blood.

7.0. 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. Standard Operation Procedures should be taken into special consideration to avoind such accidents

8.0. Recommendation

There is a need to improve on prevention control measures by improving on the existing facilities at the blood storage centres. This can in turn reduce on the risks of contamination of blood units.

There is a need for staffs who are involved in the blood collection and storage procedure to continue to adhere on their 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 and the different blood banks though the bacteria prevalence is detected in this study is very low and just skin normal flora.

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10.0 APPENDIX I

M I N I S T R Y O F H E A L T H A N D S O C I A L W E L F A R E

NATIONAL BLOOD TRANSFUSION SERVICE

Document Control # BDR 004 Version 1.0 Effective date 01/06/2010

BLOOD DONOR QUESTIONNAIRE

Donor Number: _____ **Unit Number:**

Place of Session: _____ **District:_____**

Surname:_____Other_____Names:_____

Date of Birth-----/----/------	**Age:**	**Sex:** Male /Female	**Occupation:**

Marital Status:	Not Married	Married	Divorced/Separated	Widowed

Contact Details:

Postal Address:_____Physical Address:_____Cell phone number:_____Email:_____

Donor Status	First time	Repeat	Regular	Donor club member	Donor Association

Number of donations: ____When was your last donation?____ Where?_____

1 Are you feeling well and in good health today?	Yes/No	14 In the past 6 months have you had a new sex partner?	Yes/No
2 Have you eaten in the last 4 hours?	Yes/No	In the past 12 months have you:	
3 In the past 2 weeks have you had malaria?	Yes/No	15 had a stab wound or needle stick injury?	Yes/No
In the past 6 months have you::		16 Received a blood transfusion or blood products?	Yes/No
4 been ill, received any treatment or any medication?	Yes/No	17 Had any tattooing or body piercing e.g. ear piercing?	Yes/No
5 had any operations, injections or vaccinations?	Yes/No	18 Had contact with someone with yellow eyes or yellow skin?	Yes/No
6 had typhoid?	Yes/No		
7 been pregnant or breast feeding? *(females only)*	Yes/No	19 Been a victim of sexual assault e.g. rape, sodomy or engaged in anal sex?	Yes/No
Do you have or have you ever had:		20 Had a sexually transmitted disease (STD)?	Yes/No
8 Any problems with your heart or blood pressure?	Yes/No	21 Received money goods or favors in exchange for sex?	Yes/No
9 TB or Asthma?	Yes/No		
10 Severe bleeding or a blood disease?	Yes/No	Have you ever:	
11. Sugar sickness in the blood (Diabetes)?	Yes/No	22 Had yellow eyes or yellow skin?	Yes/No
12 Any type of cancer including blood cancer?	Yes/No	23 Injected yourself or been injected, besides in a health facility?	Yes/No
13 Any other long term illness, such as epilepsy?	Yes/No	24 Do you consider your blood safe?	Yes/No

Donor Health Questionnaire

Declaration:

I confirm that the information I have given above is correct.

I consent to further communication by the BTS regarding future blood donor clinics and campaigns.

I understand and consent to my blood been tested for HIV, Hepatitis B & C, and syphilis and I am willing to receive the results of my tests from the National Blood Service.

Donor's Signature:_____ Date: _____

National Blood Transfusion Service Standard Form – NBTS Tanzania Controlled document no 04



M I N I S T R Y O F H E A L T H A N D S O C I A L

W E L F A R E

NATIONAL BLOOD TRANSFUSION SERVICE

Document Control # BDR 004 Version 1.0 Effective date 01/06/2010

BLOOD DONOR QUESTIONNAIRE

For Official Use:

Observations	**Hb** <12.5dl/L	**Hb** > 12.5dl/L	**Weight**	< 50Kg / >50Kg	**BP:** mmHg

Final Donor Assessment	Donor Accepted	Temporary deferred	Permanently deferred

Reason for Deferral	Medical	Hb	Age	B.P	Weight	Social

Polygamy	**Yes**	**No**	**Number of spouses**

Counselor Name:_____ Signature_____

Needle In	Needle Out		Unsuccessful venepuncture	Low Volume	Successful bleed
H	H				

Donor Adverse Event	Haematoma	Faint		
		Mild	Moderate	Severe

Phlebotomist Name:_____ Signature: _____

Comments:

Donor Counseling Notes:

Donor Counseled Yes / No

APPENDIX II

Procedure for performing Kirby-Bouer disk diffusion test

Procedure for performing the disk diffusion test (Kirby-Bouer method according to NCCLS 1997.

Inoculum Preparation

At least three to five well-isolated colonies of the same morphological type were selected from an agar plate culture.

The top of each colony was touched with a loop, and the growth transferred into a tube containing 4 to 5 ml of suitable broth medium, such as sterile peptone water or nutrient broth. The broth culture was incubated at 35 degree Celsius until it achieved or exceeds the turbidity of the 0.5 Mc Farland standard (usually 2 to 6 hours)

The turbidity of actively growing broth culture was adjusted with sterile saline or broth obtains turbidity optically comparable to that of the 0.5 McFarland standards.

This results in a suspension containing approximately 1 to 2 x 10^8 CFU/ml for *E.coli* ATCC25922.Using inoculums preparation, inoculums of the control organism (standard *E.coli*) was prepared.

Inoculation of the Test Plate

Optimally, within 15 minutes after adjusting the turbidity of the inoculums' suspension, a sterile cotton swab was dipped into the adjusted suspension. The swab was the rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculums from the swab.

The dried surface of the Mueller-Hinton agar plate was inoculated by streaking the swab over the entire sterile agar surface.

This procedure was repeated by streaking two more times, rotating the plate approximately 60^0 each time to ensure an even distribution of inoculums. As a final step, the rim of the agar was swabbed.

The lid was left for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks. An extreme in inoculums density was avoided. The organisms were on one plate while control organisms were inoculated on separate plate.

Application of Discs to Inocululated Agar Plates.

The predetermined batteries of antimicrobial were dispensed onto the surface of the inoculated agar plate. Each disk was placed down to ensure complete contact with the agar surface. Whether the disks are placed individually or with a dispensing apparatus, they were distributed evenly so that they are no closer than 24mm from centre to centre Ordinally, up to five discs were placed on one 100mm plate. Because some of the drug diffuses almost instantaneously, discs were not relocated once it has come into contact with the agar surface. Instead a new disc was placed in another location on the agar. The plates were inverted and placed in an incubator set to 35^0C within 15 minutes after the disc were applied.

APPENDIX III

Ethical Clearance

MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES
DIRECTORATE OF POSTGRADUATE STUDIES
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Ref. No. MU/PGS/SAEC/Vol. IV/293

November 18, 2010

Mr. Malimu, Emmanuel Zephania,
MSc. Epidemiology & Laboratory Management,
School of Public Health and Social Sciences.
MUHAS.

RE: APPROVAL OF ETHICAL CLEARANCE FOR A STUDY TITLED
"PREVALENCE OF BACTERIAL PATHOGENS IN DONATED BLOOD IN DAR ES SALAAM, TANZANIA, 2010".

Reference is made to the above heading.

I am pleased to inform you that, the Chairman has on behalf of the Senate approved ethical clearance for the above-mentioned study.

By this letter, ethical clearance is granted for you to proceed with the planned study.

Prof. Zul Premji
DIRECTOR OF POSTGRADUATE STUDIES

c.c. Vice Chancellor, MUHAS
c.c. Deputy Vice Chancellor – ARC, MUHAS
c.c. Director of Research and Publications, MUHAS
c.c. Dean, School of Public Health and Social Sciences, MUHAS

/ekl.