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Bacterial Contamination of Stored Blood Ready for Transfusion at a Referral Hospital in Ethiopia

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Abstract

Background: After the discovery of Human Immuno -deficiency virus, screening of blood donors practically reduced viral pathogens. However, transfusion associated bacterial sepsis, which causes high mortality and morbidity remain an important public health concern, has been received very little attention in the African set up including Ethiopia.

Objective: The aim of this study was to determine the prevalence of bacterial contamination of blood and their antibiotic susceptibility pattern at Debre Markos referal hospital, North West Ethiopia.

Methods: A facility based cross-sectional study was conducted using randomly sampled 120 whole blood units. The blood samples were obtained from screened, stored whole blood. All laboratory activities were carried out as Clinical and Laboratory Standards Institute (CLSI) protocol. Data were entered and analyzed using SPSS version 16 software. P<0.005 is statistically significant.

Results: The prevalence of bacterial contamination among stored blood was 12.5%. Gram positive bacteria (*S. pneumonia, S. auraus*, coagulase-negative Staphylococcus, viridian streptococcus) and gram negative (*S. typhi, E. coli* and *K. pneumonia*) were the common isolates identified.

The isolated bacterial organisms showed varying susceptibility to the antibiotics tested. All isolated gram positive organisms were resistance to Tetracycline and susceptible to Ceftriaxone. Similarly, all the gram negative organisms isolated were resistance to Cotrimoxazole and susceptible to Ciprofloxacin and Cefoxicitin

Conclusion: In our study, we conclude the existence of this serious clinical issue (contamination of blood, development of drug resistance) and need for further surveillance and/or study.

Keywords: Bacterial contamination; Sepsis; Debre Markos referral hospital

Introduction

The field of transfusion medicine has made very rapid progress after the discovery of circulation of blood in 1628 by William Harvey. Soon afterwards the first dog to dog and subsequently, lamb to human blood transfusions were attempted. During World War II and the immediate post war period the demand for blood and blood components in the USA increased substantially. This resulted in the establishment and growth of blood banks transfusion services and other blood laboratory support services [1,2].

Blood bank and transfusion services collect, process, store and provide human blood intended for transfusion [2,3]. Although, ideally blood transfusion is a safe process (i.e. that saves lives and improves the quality of life in a large range of clinical conditions), there are a number of risks associated with transfusion such viral, bacterial and parasitic infection on recipient [3,4].

Since the 1980's when the Human Immuno-deficiency Virus (HIV) was recognized, rigorous screening of blood before it is supplied to recipients was instituted [5]. Those considerable efforts (national policies, improved donor selection and newer screening techniques) directed towards reducing transmissible pathogens have yielded a major reduction of viral agents especially in developed countries [6-9].

However, transfusion transmitted bacterial infection was identified as the commonest cause of complications associated with transfusion [10]. During blood transfusion bacterial infections might be originate from the environment, from the skin of the transfused subject, or from a donor bacteremia. Most commonly, contamination occurs during blood collection (insufficient disinfection of venipuncture site), or during handling of blood products (leaky seals) [11].

The most predominant bacteria isolated are usually commensals of the skin or gastrointestinal tract flora and the majority of isolates were Gram-positive aerobic pathogens (nearly 75%) [12].

For instance, in the United States, bacterial contamination of blood accounted for 15.9% of all transfusion related fatalities [13]. However, only few countries such as Ghana [14], Uganda [15] and, Nigeria [16] in Africa have documented records of bacterial contamination of blood/blood products. For instance, in Ghana, 9-17.5% of donor bloods were contaminated by bacteria. The major bacterial isolate identified were *K. pneumoniae, E. coli, Y. enterocolitica, P. fluorescens, P. aeruginosa* and Gram positive bacteria including Bacillus species and *S. aureus* [16,17].

In Ethiopia, blood transfusion service started in 1962 and each year more than 50,000 unit of blood has been transfused [18]. Moreover, researchers in Ethiopia depicted transfusion transmissible infectious

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agents (HIV, HBV, HCV, and *T. pallidum*) have been highly prevalent among blood donors across the country and still pose a threat for public health [19-21]. For instance, Baye et al. showed that the overall prevalence of HBV, HCV and malaria parasites among blood donors in Amhara and Tigeray region were 6.2%, 1.7% and 1% respectively (20). Moreover, Tsega et al. documented that the prevalence of antibody to HCV (anti-HCV) in healthy adult Ethiopian blood donors was 1.4% [21].

To the best of our knowledge, in Ethiopia, no research has been conducted to determine blood transfusion-associated infections. Therefore, the aim of this study was to determine the prevalence of bacterial contamination of blood, to identify the types of contaminating bacteria and determine their antibiotic susceptibility pattern at Debre Markos referral hospital, North West Ethiopia.

Materials and Methods

Study design and period

A facility based cross sectional study was conducted in Debre Markos referral hospital from February 2013 to June 2013.

Sampling

Primarily we mix stored blood and randomly sampled 120 units of blood from storage which was collected in Debre Markos referral hospital blood bank. The blood samples were obtained from screened, stored whole blood. All expired blood was 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. 5ml of blood was drawn from the closest end to the bag with a sterile syringe and needle and dispensed into 15ml of Brain-Heart Infusion (BHI) broth [22].

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 [22].

Antibiotic susceptibility testing

Selection of antibiotics is based on current treatment regimen for gram positive and negative. Susceptibility to antimicrobial agents was tested by the disc diffusion technique according to the guidelines by the Clinical and Laboratory Standards Institute (CLSI). 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 and rifampicin 5 µg (Oxoid). The discs were placed on to the surface of inoculated Mueller- Hinton agar plates by an auto dispenser. 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. *E. coli* ATCC 25922 was used as the control strain [23-27].

Statistical analysis

Data were entered, cleaned and analyzed using SPSS (Statistical Package for Social Science) version 16 by a trained data encoder. P values<0.05 were statistically significance.

Ethical considerations

Ethical clearance was obtained from Research and Publication Directorate Office of Debre Markos University, Ethiopia. Support letter was sent to Debre Markos referral hospital. Moreover, written consent was obtained from the hospital administration and laboratory head. All the data were recorded using codes, no name and other personnel identification. The result obtained in this research was confidential and only the result output disseminated for concerned bodies.

Result

Bacterial isolate and their susceptibility pattern

The length of storage of the blood ranged from 0 to 12 days (mean=4 days), and most of the contaminated samples (73.3%) had <1 week of storage (Figure 1). Of the 120 samples tested 15(12.5%) were found to be contaminated with various types of bacteria and making a 12.5% prevalence of bacterial contamination of whole blood at the Debre Markos and Fenote Selam hospital (Table 1).

Gram positive bacteria (*S. pneumonia, S. auraus, coagulase-negative Staphylococcus, viridian streptococcus*) and gram negative (*S. typhi, E. coli and K. pneumonia*) accounting 66.7 and 33.3%, respectively, were the common isolates identified (Table 1).

The two leading whole blood contaminant isolate were *S.*

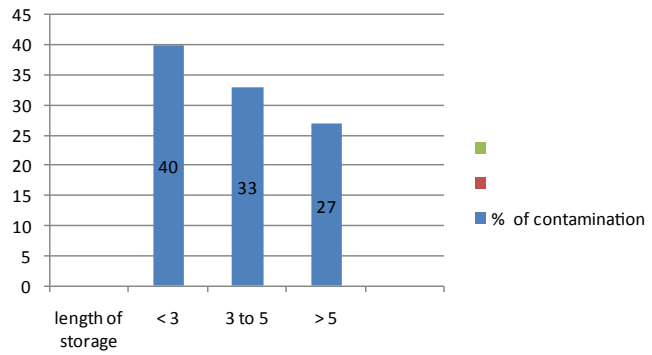

Figure 1: The length of storage of whole blood samples and percentage of bacterial contamination at Debre Markos referral hospital, North West Ethiopia, February 2013 to June 2013.

Microorganism	Time of storage
S. pneumonia	Day 0
Viridian streptococcus	Day 5
Viridian streptococcus	Day 2
Viridian streptococcus	Day 4
S. pneumonia	Day 0
S. auraus	Day 4
S. auraus	Day 3
S. auraus	Day 2
Coagulase negative staphylococcus	Day 5
Coagulase negative staphylococcus	Day 4
K. pneumonia	Day 8
S. typhi	Day 0
E. coli	Day 9
E. coli	Day 10
K. pneumonia	Day 12

Table 1: The isolated microorganisms and time of storage at Debre Markos and Fenote Selame hospital, North West Ethiopia, February 2013 to June 2013.

Isolate	Antibiotics															
	Ciprofloxacin	Ceftrixon	Norfloxacin	Chloramphenicol	Co-trimoxazol	Oxaciline	streptomycin	vancomycin	Tetracycline	Cefoxicitin	erythromycin	Rifampin	Amoxaciline	Clindamycin	Augumentin	Naldixic
S. auraus (n=3)	NT	NT	NT	IRS	IRR	SRI	SSS	IRS	RRR	SSI	ISS	RRI		SSI	NT	NT
CoNS (N=2)	NT	NT	NT	NT	IR	SS	NT	SS	RR	SS	IR	NT		NT	NT	NT
Viridian streptococci (n=3)	NT	NT	NT	SSSS	SIIII	SIISR	NT	IRIRR	RRRRR	SSSSS	IISSS	NT		IISSS	SIISS	RRRRI
S. typhi (n=1)	SSSSS	SSSSS	IIRRRR	IIIRS	RRRSR	SIIII	NT	NT	IIIRR	NT	NT	NT	IIRRR		SIIII	NT
K. pneumonia (n=2)	SSSSS	SSSSS	IIRRRR	IIIRS	RRRSR	SIIII	NT	NT	IIIRR	NT	NT	NT	IIRRR		SIIII	NT
E. coli (n=2)	SSSSS	SSSSS	IIRRRR	IIIRS	RRRSR	SIIII	NT	NT	IIIRR	NT	NT	NT	IIRRR		SIIII	NT
S. pneumonia (n=2)	NT	NT	NT	SSSS	SIIII	SIISR	NT	IRIRR	RRRRR	SSSSS	IISSS	NT	NT	IISSS	SIISS	RRRRI

R-resistance, I-intermediate, S-susceptible, NT-not done

Table 2: Antibiotic resistance pattern of the bacteria isolated from stored blood at Debre Markos referral hospital, North West Ethiopia, February 2013 to June 2013.

pneumonia 5/15(33.3%) and *S. typhi* 5/15(33.3%). The isolated bacterial organisms showed varying susceptibility to the antibiotics tested (Table 1). All isolated gram positive organisms were resistance to Tetracycline and susceptible to Cefoxiciti. Similarly, all the gram negative organisms isolated were resistance to Cotrimoxazole and susceptible to Ciprofloxacin and Cefoxicitin (Table 2).

No significant difference was observed among the blood group O (10/60, 16.7%), blood group A (2/22, 9.1%), and blood group B (3/30, 10%) P>0.005, data not shown).

Discussion

Knowledge of the prevalence of bacterial contamination of blood for transfusion and the sources or the causes of contamination is important for the planning of preventive measures at blood transfusion centers and the reduction of transfusion transmitted bacterial infections. 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. It is also important to provide the basis for action and changes in blood transfusion practices, policy, and education [28,29].

However, the problem of bacterial contamination of blood has been received very little attention in the African set up, only few countries such as Ghana [14,24] , Uganda [15], Nigeria [16] and Kenya [23] have published records of bacterial contamination of blood or blood products.

In the present study, we report a prevalence of 12.5%, which is higher than a similar study conducted in Uganda 3.5% [15], Nigeria 8.8% [16], Kenya 7% [23], United State 0.2% [25], United Kingdom 0.15% [26] and in France 0.1% [27]. The possible explanation could be the difference in the blood transfusion laboratory set up across the countries and attribute to implementation of more rigorous screening procedures practiced in the blood transfusion centers.

The observation of the increased prevalence of bacterial contamination of donor blood raises concern about the need for preventive measures such as systemic and comprehensive donor selection and screening, scrubbing of the phlebotomy sites with improved disinfectants, and improved screening tests, as well as culturing of donor blood, particularly for immune compromised individuals.

The organisms isolated in this study were both gram positive (*S. auraus, coagulase negative staphylocci, viridian streptococcus, S. pneumonia*) and gram negative (*K. pneumonia, S. typhi and E. coli*). Our finding was in agreement with study conducted in Uganda [15], Nigeria [16] and Kenya [23].

In this study, we depicted that the contamination of blood by gram positive organisms were documented within a week while contamination of blood by gram negative organisms were somewhat delayed. Our find was in agreement with different studies which claimed that Gram-positive commensals are isolated soon after donation, whereas Gram negative organisms not usually detectable until after a period of proliferation during storage [30-32].

Moreover, in contrast to other previous studies conducted elsewhere, in the present study both *S. pneumonia and S. typhi* were isolated at day=0 and this is an indication of bacterial contamination will be arising from donor bacterimia. As a result, efforts or measures to ensure blood transfusion safety such as adequate cleaning of phlebotomy sites, improved donor selection and screening should be strengthened.

In our finding, high rate of drug resistance for both gram negative and positive isolate were observed. These organisms might be cause septicemia and serious risk of fatality after post-transfusion takes place. Similarly, this high rate of drug resistance highlights the growing problem of antimicrobial resistance worldwide. Our finding was consistent with studies conducted in Ghana [14] and Uganda [15] and other parts of the world [31,33]. The possible explanation for the high resistance of donor blood isolates may be associated with the ease of procuring antibiotics, self medication and inefficient infection control procedure across the country.

Conclusion and Recommendation

Our result indicated that the prevalence of bacterial contamination of stored blood ready for transfusion at Debre Markos was high. In addition, high rate of drug resistance was observed for isolated bacterial strains. Therefore, preventive measures such as systemic and comprehensive donor selection and screening, scrubbing of the phlebotomy sites with improved disinfectants, and improved screening tests, as well as culturing of donor blood, particularly for immune compromised individuals should be carried out.

Limitation of the Study

The small sample size and our inability to follow up the recipients of the blood units to determine clinical outcome of infection may be some of the limitation of this study. In addition, we enrolled only whole blood since blood component separation does not undertaken in the hospital

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Competing Interests

This work was sponsored by Debre Markos University, Ethiopia. No financial aid was received from any organization for publication or other interest. There is no any competing of interest.

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