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Original Article

Bacterial Contamination of Blood and Blood Components in Three Major Blood Transfusion Centers, Accra, Ghana

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SUMMARY: Reports from studies conducted in several countries indicate a high incidence of bacterial contamination of donor blood. The prevalence of bacterial contamination of blood and its products in Ghana is not known. This study was conducted to determine the prevalence of bacterial contamination of blood and its products at the three major blood transfusion centers in the Greater Accra Region of Ghana. Stored whole blood and its products were cultured on different media, and isolates were identified using standard biochemical and bacteriological methods. The susceptibility of the isolates to selected antimicrobial agents was also determined by the disc diffusion method. The overall prevalence rate was 9% (28/303; whole blood, 13% [24/192]; plasma, 3% [2/79]; platelet, 9% [2/22]). The Gram-positive bacteria isolated were coagulase-negative *Staphylococcus*, *S. aureus*, and *Bacillus* spp., and the Gram-negative organisms were *Yersinia enterocolitica*, *Citrobacter freundii*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. The Gram-positive bacteria were sensitive to cloxacillin, erythromycin, tetracycline, and gentamicin but resistant to penicillin, ampicillin, cefuroxime, and cotrimoxazole, while the Gram-negative bacteria were sensitive to amikacin and gentamicin but resistant to chloramphenicol, tetracycline, ampicillin, cefuroxime, cefotaxime (except *Y. enterocolitica*), and cotrimoxazole. Our results suggest that bacterial contamination of blood and its products is prevalent in Ghana.

INTRODUCTION

The relation between blood transfusion and the high transmission of certain bacterial and viral infectious diseases, such as syphilis, diseases caused by *Streptococcus* spp., tuberculosis (TB), and human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), and human T-cell lymphotropic virus (HTLV) infections, among blood recipients have been known for several years (1-3). For the past three decades, attention has been focused on the risk of transmission of viruses through the transfusion of blood and blood components. Considerable progress has been made in this area, and the transmission of viruses such as HIV, HBV, HCV, and HTLV has been greatly reduced due to the introduction of improved donor selection and improved screening tests (4-6). In contrast, despite the introduction of newer donor selection methods and improved screening tests for transfusion-transmissible diseases, transfusion-transmitted bacterial infection has been identified as the most common and severe infectious complication associated with transfusion (7,8). Approximately 57% of all transfusion-transmitted infections and 16% of transfusion-related deaths have been associated with bacterial contamination (7). In the United States, bacterial contamination of blood accounts for as many as 500 to 750 deaths annually (9), and between 1986 and 1991, bacterial contamination accounted for 15.9% of all transfusion-related fatalities (10). In France, the 'Haemovigilance' surveillance system of the French Blood Agency attributed 18 deaths between 1996 and 1999 to bacterially contaminated blood components (11). The bacteria implicated in the transfusion of blood and its products are Gram-negative bacilli such as *Yersinia enterocolitica*, *Pseudomonas fluorescens*, and *Pseudomonas aeruginosa*. Other species are Gram-positive species including *Staphylococcus* and *Streptococcus* spp. (12-14).

Blood and blood components may be exogenously contaminated as a result of defective blood bags or collection equipment, by contamination of the blood at the time of collection or during processing or storage (15,16). Collected blood may also be endogenously contaminated as a result of an asymptomatic bacteremia in the donor or as a result of inadequate disinfection of the venepuncture site of the donor (17,18). Bacterial contamination and its associated transfusion infections has been reduced tremendously in developed countries because blood donors are carefully screened by questions related to bacterial and parasitic infections (19). Phlebotomy sites of donors' skin are carefully prepared using improved skin disinfection methods (20,21). All erythrocyte units and platelets units are cultured or leukodepleted to further remove bacteria from blood components before transfusion (22). In contrast, less developed countries, including Ghana, are not able to fully implement the above procedures to ensure the safety of transfused donor blood. Also, there is no systematic and comprehensive donor selection screening for bacterial and parasitic pathogens in many of the blood transfusion centers in the country. In almost all the regional and district health care facilities with blood transfusion services in the country, donor blood is screened mostly for HIV, HCV, HBV, and sometimes syphilis. A questionnaire relating to bacterial and parasitic infections and high-risk

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behaviors is the only line of protection against certain infections like malaria, leishmaniasis, *Y. enterocolitica*, *P. fluorescens*, and *Staphylococcus* and *Streptococcus* spp. for which no testing is performed. Such a questionnaire is sometimes not fully applied and in some instances is not administered at all. Although the prevalence of severe episodes of transfusion-associated bacterial sepsis has not been clearly established in Ghana, anecdotal reports suggest that the prevalence or incidence of bacterial sepsis as a result of transfusion of contaminated blood or its products is high. Taking into consideration the high demand for blood transfusion service, the high endemicity of infections, and high fatalities from bacterial sepsis, we investigated the prevalence of bacterial contamination of blood and blood components in three of the major transfusion centers in the Greater Accra Region of Ghana.

MATERIALS AND METHODS

Study design and study site: The study was designed as a multicentre cross-sectional study and was conducted between February 2008 and July 2008 among a random sampling of stored whole blood and processed blood (plasma, platelets, and cryoprecipitate) at three of the major blood donation and transfusion centers (the 37th Military Hospital Blood Transfusion Unit [MHBTU], the Ridge Hospital Blood Transfusion Unit [RHBTU], and the National Blood Transfusion Service [NBTS]) all in the Greater Accra Region of Ghana.

The NBTS, located at the Korle-Bu Teaching Hospital (KBTH), Accra, Ghana, serves the KBTH (a tertiary and leading referral hospital in Ghana) and other hospitals/clinics in the Accra (capital city of Ghana) metropolis. It also serves both governmental and non-governmental institutions, and transfusion centers in the central and eastern regions of Ghana.

The 37th Military Hospital, located about 15 km from the NBTS, serves mostly military personnel of the Ghana Armed Forces and their dependents. It also serves the surrounding urban population and referred cases of other military personnel from other countries on a peace-keeping mission in the West African Sub-Region. The Ridge Hospital, also based in Accra (about 5 km from NBTS), was originally built to primarily serve personnel and dependants of the then colonial British administration. Following independence and the exit of the colonial powers, the hospital by convention became the Greater Accra regional hospital and currently serves all inhabitants of the region and elsewhere.

Only samples collected, screened, and stored (up to 5 weeks) for transfusion purposes at the selected centers were used. This included samples obtained from voluntary donors (both outreach programs and those donated within the centers) and replacement donors at the above centers. Samples that tested positive for transfusion-transmitted infections (HIV, HBV, HCV, and syphilis) and expired blood samples were not included in the study.

Blood and blood product collection: A total of 303 blood bags were randomly selected for the study. All sample collection and testing procedures were done using standard bacteriological safety and aseptic procedures. Maximum safety measures required for the collection and handling of blood and bacteriological samples were adhered to and employed. Disinfection was done using 70% ethanol. Stored blood in bags was thoroughly mixed, and the end of the tied tubing was then swabbed with disinfectant and cut with sterile scissors to discard clotted blood (if any) in the line; some of the mixed blood from the main bags was allowed to seep into the line. The end of each line was then clipped with Spencer forceps to prevent blood in the line and air from flowing back into the main bag. Three knots were made for each bag, and the last knot was swabbed with 70% ethanol, punctured with a sterile syringe and needle to draw approximately 1 ml of sample (stored whole blood, processed blood [platelets, plasma, and cryoprecipitate]) and dispensed separately into 9 ml of sterile brain-heart infusion (BHI) broth.

Culturing and identification: Each of the sample suspensions was incubated at 37°C for 7 days and observed daily for any possible signs of bacterial growth (pellicle formation, hemolysis, turbidity). After an overnight (24 h) incubation, a sterile loop full of each broth suspension was subcultured onto blood agar (BA), chocolate agar (CA, in a candle jar), and MacConkey agar (MA) plates and incubated overnight (18 - 24 h) at 37°C. Any bacterial growth was identified using colonial morphology, Gram stain reaction, standard biochemical and sugar fermentation tests, and the Mini API 20E identification tests (BioMeriuex, Marcy-L'Etoile, France).

Antibiotic susceptibility testing: The susceptibility of the isolates to selected antimicrobial agents was determined by the disc diffusion method and the Kirby Bauer method (23) using antibiotics-impregnated paper discs (Medical Wire & Equipment Co. Ltd., Potley Corsham, England) containing the following antibiotics: amikacin (AMK, 30 μg), gentamicin (GEN, 10 μg), ampicillin (AMP, 10 μg), penicillin (PEN, 10 μg), tetracycline (TET, 30 μg), erythromycin (ERY, 15 μg), chloramphenicol (CHL, 10 μg) cotrimoxazole (COT, 25 μg), cefuroxime (CXM, 30 μg), cloxacillin (CXC, 5 μg), and cefotaxime (CTX, 30 μg). Pure colonies of fresh isolates were emulsified in peptone water using a straight sterile wire, and the turbidity was adjusted to the equivalent of 0.5 MacFarland standard. A sterile cotton swab was then used to obtain a portion of the emulsified suspension to make a three-dimensional (3D) streak on a Mueller Hinton agar plate. An appropriate antibiotic disc (i.e., either Gram-negative or -positive multidisc plus antibiotic) based on the organism's Gram reaction was then placed on the plated agar lawn within 15 min of seeding and then incubated at 37°C overnight (18 - 24 h). The diameter of the zone of inhibition was determined using a ruler and compared with a standard chart to determine susceptibility (sensitive or resistant). Control strains used for the test were *Escherichia coli* (NCTC 10418) for Gram-negative organisms and *Staphylococcus aureus* (NCTC 6571) for Gram-positive organisms.

Statistical analysis: The EPI-INFO 2005 statistical software package (Centers for Disease, Control and Prevention, Atlanta, Ga., USA) was used to complete all data analyses. The chi-square test was used to determine any statistically significant differences between the transfusion centers. $P < 0.05$ was considered significant.

RESULTS

Between February and July 2008, 303 samples (whole blood, platelets, plasma, and cryoprecipitate) were randomly selected from MHBTU (whole blood, 60; platelets, 10; plasma, 39), RHBTU (whole blood, 59; platelets, 0; plasma, 0), and NBTS (whole blood, 73; platelets, 12; plasma, 40; cryoprecipitate, 10) for the study. Of the 303 samples tested, 28 (9%) were found to be contaminated with various types of bacteria.

Table 1 shows the level of contamination of the various blood products. Whole blood had a significantly higher ($P < 0.05$) level of bacterial contamination compared to the rest of the blood products (platelets, plasma, and cryoprecipitate) in the three major transfusion service centers.

As shown in Table 2, among the three transfusion centers, MHBTU had a greater number ($P < 0.05$) of whole blood samples contaminated with various types of bacterial organisms than were found in the whole blood samples obtained from NBTS and RHTBU.

Gram-positive bacteria (*S. aureus*, coagulase-negative *Staphylococcus*, and *Bacillus* sp.) and Gram-negative bacteria (*Y. enterocolitica*, *Citrobacter freundii*, *E. coli*, *P. aeruginosa*, and *Klebsiella pneumoniae*), accounting for 53 and 47%, respectively, were the common isolates identified (data not shown).

The isolated bacterial organisms showed varying susceptibility to the antibiotics tested (Table 3). All the isolated Gram-positive organisms were resistant to CXM, PEN, AMP, and COT but sensitive to CXC, TET, ERY, and GEN. Similarly, all the Gram-negative organisms isolated were resistant to CTX (except *Y. enterocolitica*), TET, AMP, CXM, COT, and CHL but sensitive to AMK and GEN.

Of the blood group types, blood Group O had significantly higher (20%, 14/69) prevalence of bacterial contamination compared to blood Group A (8%, 3/39), blood Group B (11%, 6/54), and blood Group AB (3%, 1/30), ($P < 0.05$).

Table 1. Levels of contamination of blood and its products

	Blood bags tested	No. contaminated (%)
Whole blood	192	24 (13)
Plasma	79	2 (3)
Platelet	22	2 (9)
Cryoprecipitate	10	0 (0)
Total	303	28 (9)

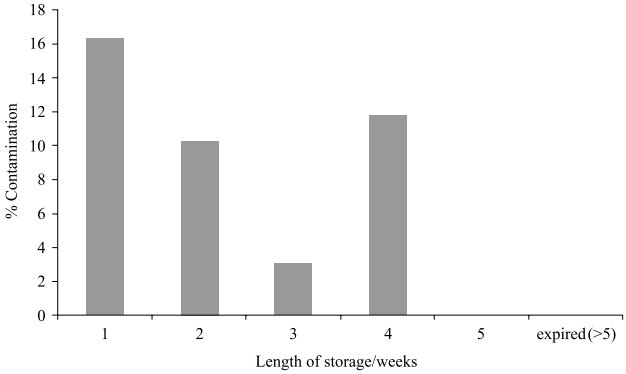

Fig. 1. Length of storage of blood and % contamination. One week of storage of blood samples; total number of blood samples, 98. Total number of bacteria contamination, 17. Two weeks of storage of blood samples; total number of blood samples, 39. Total number of bacteria contamination, 4. Three weeks of storage of blood samples; total number of blood samples, 33. Total number of bacteria contamination, 1. Four weeks of storage of blood samples; total number of blood samples, 17. Total number of bacteria contamination, 2. Five weeks of storage of blood samples; total number of blood samples 3. Total number of bacteria contamination, 0.

The length of storage of whole blood samples and percentages of bacterial contamination are shown in Figure 1. In all the three centers, whole blood stored for a period of 1 week had the highest levels of bacterial contamination ($P < 0.05$) compared to whole blood samples stored for 5 weeks.

DISCUSSION

Knowledge of the prevalence of bacterial contamination of blood and its components for transfusion and the sources or causes of contamination in different parts of the world, particularly in Africa, is important for the planning of preventive

Table 2. Number of blood and its products contaminated with bacteria at the blood transfustion centers

	MHBTU		RHBTU		NBTS		*P*
	No. tested	No. postive (%)	No. tested	No. positive (%)	No. tested	No. positive (%)	
Whole blood	60	10 (17)	59	7 (12)	73	7 (10)	<0.05
Plasma	39	1 (3)	0	0 (0)	40	1 (3)	>0.05
Platelet	10	1 (10)	0	0 (0)	12	1 (9)	>0.05
Cryoprecipitate	10	0 (0)	0	0 (0)	10	0 (0)	>0.05

MHBTU, 37th Military Hospital Blood Transfusion Unit; RHBTU, Ridge Hospital Blood Transfusion Unit; NBTS, National Blood Transfusion Service.

Table 3. Antibiotic susceptibility to bacteria isolated from the contaminated samples at the blood transfusion centers

	Antibiotic										
	CTX	ERY	GEN	TET	CXC	PEN	AMP	CXM	COT	CHL	AMK
Gram-positive bacteria											
Bacillus sp.	–	SEN	SEN	SEN	SEN	RES	RES	RES	RES	–	–
CNS	–	SEN	SEN	SEN	SEN	RES	RES	RES	RES	–	–
Staphylococcus aureus	–	SEN	SEN	SEN	SEN	RES	RES	RES	RES	–	–
Gram-negative bacteria											
Klebsiella pneunoniae	RES	–	SEN	RES	–	–	RES	RES	RES	RES	SEN
Escherichia coli	RES	–	SEN	RES	–	–	RES	RES	RES	RES	SEN
Yersinia enterocolitica	SEN	–	SEN	RES	–	–	RES	RES	RES	RES	SEN
Citrobacter freundii	RES	–	SEN	RES	–	–	RES	RES	RES	RES	SEN
Pseudomonas aeruginosa	RES	–	SEN	RES	–	–	RES	RES	RES	RES	SEN

CTX, cefotaxime; ERY, erythromycin; GEN, gentamicin; TET, tetracycline; CXC, cloxacillin; PEN, penicillin; AMP, ampicillin; CXM, cefuroxime; COT, cotrimoxazole; CHL, chloramphenicol; AMK, amikacin; SEN, sensitive; RES, resistant; CNS, coagulase-negative *Staphylococcus*.

measures at blood transfusion centers and the reduction of transfusion-transmitted bacterial infections. Furthermore, determination of the type(s) of bacterial organisms involved and identification of the sources and risk factors among the blood donors and the staff involved in the processing and treatment of donor blood are important to provide the basis for action and changes in blood transfusion practices, policy, education, and clinical practice. The purpose of this study was to determine the prevalence of bacterial contamination of blood and its products at three major transfusion centers in the Greater Accra Region of Ghana.

The present study, which is believed to be the first in the country, provides definitive evidence of bacterial contamination of transfused blood occurring within our major blood transfusion centers. The results show a significantly high percentage of bacterial contamination of whole blood in MHBTU (17%), RHBTU (12%), and NBTS (10%). The overall rates in the three major transfusion centers are higher than the rates (0.2, 0.1, and 0.15%) detected in the United States (24), France (25), and the United Kingdom (26,27), respectively. The observation of the increased prevalence or percentage of bacterial contamination of donor blood raises concerns about the need for preventive measures, such as systematic 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 immunosuppressed individuals. The type of bacterial organisms isolated and identified from the contaminated blood samples in the three major transfusion centers are consistent with reports in the literature (28-30). The source(s) of contamination could not be discerned from our study. However, this observation may be due to donor bacteremia, inappropriate work-related behaviors practiced in the transfusion centers, poor storage conditions, and/or inadequate disinfection of the venepuncture site of the donor. Further studies need to be done to clearly define the sources of bacterial contamination of blood and its components in the transfusion centers and the selection of blood donors.

Strikingly, most of the organisms isolated and identified in our study are resistant to currently used antibiotics in the country (31,32) and elsewhere (33). These organisms cause serious risk of fatality when transfused into immunosuppressed patients (25).

Another finding of interest reported herein is the length of storage of blood and the prevalence of bacterial contamination. Because bacteria strains often proliferate in blood and its products during storage, some consideration has been given to shortening storage times (at least 2 weeks at 1°C to 6°C) in the hope of reducing transfusion-associated sepsis (8, 34,35). However, in our study, whole blood samples stored up to 1 week at 4°C recorded the highest levels of bacterial contamination (Figure 1). The reason(s) for this disparity cannot be discerned from our study. However, we observed during our study period that refrigerators employed for the storage of blood samples were frequently opened and also used for the storage of other reagents. Moreover, neither the various types of equipment and instruments used in processing of blood for transfusion nor the water bath used for thawing of blood samples was regularly sterilized. Further studies need to be done to clearly characterize the length of storage of blood samples and the contribution of storage time to bacterial contamination in the transfusion centers in the country.

The small sample size, particularly plasma and platelets, and our inability to follow recipients to ascertain whether they developed septicemia as a result of the transfusion of contaminated blood may be some of the limitations of this study. The detection of contamination of donor blood with bacterial organisms in the three major transfusion centers in the Greater Accra Region may reflect the situation among transfusion centers in the country. Further studies need to be done to define clearly the prevalence of bacterial contamination among transfusion centers in Ghana to effectively control and prevent contamination of donor blood and its products.

The results reported herein have significant implications for blood transfusion managers, persons who work at the transfusion centers, public health officials, and clinicians, and suggest an urgent need for the introduction of policies for safe transfusion practices and education among blood donors and staff members at the centers. This is necessary because the demand for blood in Ghana is high as a result of excessive incidence of infections that cause anemia, malnutrition, surgical emergencies including road traffic accidents, and obstetrical emergencies associated with blood loss.

In conclusion, our results suggest that bacterial contamination of donor blood and its products may be prevalent in the blood transfusion centers in the Greater Accra Region of Ghana and that persons who received blood in the three major centers are at potential risk of developing infection. The high prevalence of contaminated blood in the three major transfusion centers in Ghana suggests the urgent need for the introduction of some of the range of effective preventive strategies employed in blood transfusion centers elsewhere.

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