

Bacterial Contamination of at-Point-of Transfusion Blood in a Tertiary Hospital in Ghana

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Abstract

Background: Hand-in-hand demand for blood transfusion and transfusion-related bacterial infections among blood recipients in Sub-Saharan Africa have increased. Due to resource constraints and increased demand for blood donations in Sub-Saharan Africa, it is a common practice at some blood transfusion centers, where after initial screening of blood and its storage, the stored blood (non-expired blood) is not rescreened at least for bacterial contamination prior to transfusion, a practice which has the potential to expose blood recipients to high risk of bacterial sepsis, especially if blood were donated at window period of contaminating pathogens. This study investigated bacterial contamination of whole blood at-point-of-transfusion at a referral hospital in the western region of Ghana.

Method: Pre-screened and stored blood units about to be sent out for transfusion to recipients at patient wards were purposively sampled, and cultured in different culture media (brain heart infusion broth, blood agar, Mackonkey agar, and plate count agar). Colonial morphology, Gram stain reactivity, and standard biochemical and bacteriological methods were used to identify bacteria isolates. Standard plate count was used to enumerate growth of each bacteria isolate.

Results: From 97 blood units sampled, 16 (16.5 %) were blood contaminated (30-300 cfu/ml) with Gram-negative (*E. coli*, *Pseudomonas aeruginosa*, and *Enterobactersp*) and Gram-positive (*Bacillus sp*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*) bacteria species. A total of 16 bacteria isolates comprising Gram-negative (43.8 %) and Gram-positive (56.2 %) were identified.

Conclusion: Bacterial contamination of at-point-of-transfusion may be common in the study area, and while this observation may stimulate further investigations, local hospital-constituted transfusion oversight committees as well as national, regional, and district health authorities must increase monitoring, supervision, and enforcement of standard sterile and aseptic procedures at all blood transfusion centers nation wide.

Keywords: Bacterial contamination; Blood transfusion; Ghana; Stored blood

Introduction

Bacterial contamination of whole blood and blood components, and the specific bacteria commonly implicated are either not reported or under-reported in Sub-Saharan Africa [1,2] despite global increase in episodes of sepsis and other transfusion-related complications

from transfusion of bacteria contaminated blood [3-5]. In developed countries virus-related transfusion-transmissible infections and complications have been curtailed almost completely, due to stricter donor selection criteria, advanced screening methods, skilled personnel, strict adherence to standard sterilization and aseptic procedures at all stages of blood transfusion processes [6, 7], nonetheless, bacterial contamination of blood remains a challenge in those settings [8]. Example, mortality (16 %) and post-transfusion septic episodes (57 %) among blood recipients in developed countries were attributed to bacterial contamination of blood products [8]. In USA, 500-750 patients lose their lives as a result of bacterial contamination of blood [9].

Expectedly, the problem of bacterial contamination of blood products and its attendant complications are getting worse in Sub-Saharan Africa, where standard screening methods, trained personnel and blood testing facilities are major constraints [10]. Like in developed countries, screening of viral blood contaminants (HIV, HBV, HCV, HTLV) has steadily improved with the recent adoption of improved pre-transfusion checks, procedures and guidelines in some developing countries including Ghana, however, risk of bacterial contamination remains a major health threat [11] in view of the relatively inadequate resources. In Sub-Saharan Africa demand for blood donations and transfusion services have significantly increased, partly due to increased incidence of infectious diseases, malnutrition in children, anemia in pregnant mothers and children, increased emergency cases from surgicals, obstetrics and road accidents [12-15] and indiscriminate prescription of blood and its components to patients [16]. These factors have resulted in increased pressure on scarce blood screening and testing facilities as well as the quality of donor blood transfused to prospective recipients. Example, the closed system of blood units is commonly breached through cross-matching/re-cross-matching, division of blood units into smaller volumes, especially for children [1]. Also, use of donor selection procedures (questionnaires) which are fraught within correct and unreliable responses from voluntary donors [12] and use of observational method (physical changes in blood color and flow) to determine bacterial contamination of ready-to-be-transfused blood instead of blood culture [10,17]. Further, contaminated blood bags/equipment, poor venipuncture practices, use of improper aseptic agents (use of alcohol instead of chlorhexidine), poor blood processing practices, and storage [18,19], low sensitivity of screening methods [11], exclusion of some infectious pathogens [17,20,21], and collection of donor blood at window period of infectious agents [22,23] are common risk factors for bacterial contamination in Sub-Saharan Africa. Of note, septic episodes among blood recipients secondary to blood transfusion has been linked to many bacteria contaminants, including skin flora [24], *Staphylococcus aureus*, *Staphylococcus epidermis*, *Micrococcus sp*, *Corynebacterium sp* [25-27]; *Bacillus sp*, *Yersinia enterocolitica*, *Enterobacter aerogenes*, *Serratia liquefaciens*, *Campylobacter jejuni*, *Enterobacter sp*, *Flavobacterium*, and *Salmonella sp* [28,29].

In Ghana, efforts have been made for the past decade to raise not only awareness about blood donations, but also establishment and proper management of blood banks in all the ten regions of the country, through the medium of supervision, public education and research efforts, nonetheless bacterial contamination of blood remains common [12,17,20]. The quality (contamination-free blood) of at-point of transfusion blood is critical to the health and post-transfusion health outcome of blood recipients, but the status of these important aspects of the blood transfusion process remains poor in Ghana. Although, previous studies in Ghana have investigated the quality of transfusion services and hazards of some blood transfusion centers [12,17,20,30], many other blood transfusion centers in other regions remain unattended to. This study investigated the prevalence of bacterial contamination of whole blood at the point-of-transfusion at a referral hospital in the Western region of Ghana.

Materials and Methods

Study Area

The study was conducted at the medical laboratories of Efiya-Nkwanta Regional Hospital (ENRH) in Sekondi-Takoradi Metropolis (STM), the capital city of the western region of Ghana. ENRH is the largest hospital in the western region where most of the population seeks medical care. There are other hospitals including Takoradi Hospital (TH), Kwesimintsim Hospital (KH) and Essikadu Hospital (ESKH) which also offer healthcare services but often refer emergency cases to ENRH. The study was conducted from December, 2011 to April, 2012. STM is the administrative capital of the Western Region of the Republic of Ghana. It covers a land area of 385 km² with Sekondi as the administrative headquarters. STM is bordered to the West by Ahanta West District, to the North by MponohorWassa East, to the East by Komenda-Edina Eguafo-Abrem and to the South by the Gulf of Guinea (Ghana Statistical Service, 2012). The Metropolis is located on the

south-western coast of Ghana, about 200 km west of Accra and 130 km East of La Cote D'Ivoire (Sekondi-Takoradi Metropolitan Assembly, 2013). STM is a densely populated district having the largest share (23.5 %) of the region's total population of 2,376,021. The current population of the Metropolis stands at 559,548 with 273,436 males and 286,112 females (Ghana Statistical Service, 2012). The central part of the Metropolis is low lying and occupied by muddy lagoons. It experiences a bi-modal type of rainfall. With a mean annual rainfall of 1,380 mm, the metropolis experiences heavy rainfall in March and July with minor rains occurring between August and November (Ministry of Food and Agriculture, 2013). Temperatures are uniformly high with an average of 22°C (Ministry of Food and Agriculture, 2013).

Sample

Blood samples collected (from voluntary and replacement donors) pre-screened (for virus-and-bacteria contaminants), stored (≤ 3 weeks) and about to be transfused to recipients were purposively sampled.

Sample collection

A total of 97 blood packs were purposively sampled from the blood bank of ENRH after they have been cleared for transfusion. The collection and sample preparations were done as previously described [12]. Briefly, after thorough mixing of stored blood in bags, the tied end of each tubing was disinfected and cut open by using a sterile scissor to get rid of blood clots where applicable. The well mixed blood from each bag was carefully made to gently flow in the cut tubing. Subsequently, the cut end of the tubing was tightly clipped by using a forceps, followed by tying of 3 knots along the line. After disinfecting the last knot with 70 % ethanol, a sterile syringe and needle were used to draw 1 ml of whole blood into each universal culture bottle containing 9 ml of an already prepared sterile brain-heart infusion broth. Standard safety and aseptic measures required for the collection, handling and processing of blood and microbiological samples were strictly followed.

Incubation

Each sample (stored whole blood [1 ml] + brain heart infusion broth [9 ml]) in universal culture bottles was aerobically incubated for 7 days at 37°C and checked every 24 h for bacteria growth (turbidity, pellicle formation and hemolysis) as previously described [12].

Sub-culturing

After 24 h of incubation, a microbiological loop sterilized by flaming until red hot and cooled by swirling in air was inserted into each sample and sub-cultured onto Blood Agar (BA) and MacConkey Agar plates and aerobically incubated for 18-24 h at 37° C. After incubation both plates were examined for bacteria growth.

Identification of Bacterial Isolates

Colonial morphology was first used to identify colonies followed by Gram stain reactivity and standard biochemical (Indole test, Catalase test, Coagulase test, Urease test, Citrate test, Oxidase test) and Triple Sugar Iron test (sugar fermentation and gas elimination) to finally confirm identity of bacteria isolates.

Standard Plate Count of Isolates

Each sample (stored whole blood [1 ml] + brain heart infusion broth [9 ml]), after 24 h incubation was serially diluted as follows. One milliliter of each sample was transferred into 9 ml sterile saline in a tube and thoroughly mixed, representing a 1/10 serial dilution. Subsequently, sequential dilutions were made up to 1/10000. A 0.1 ml of each serial dilution for each isolate was plated on standard size petri dishes with Plate Count Agar (PCA). After 24 h incubation at 37° C, plates with 30-300 CFU were selected and enumerated.

Statistical Analysis

Results were presented in tables using descriptive statistics (mean and frequency of occurrence expressed in percentages [%]).

Results

Out of 97 blood units sampled and tested for significant (30-300 cfu) bacteria growth, 16.5 % (16/97) were found to be contaminated

with bacteria. In all sixteen bacteria isolates were identified comprising three each of Gram-negative (*E. coli*, *Pseudomonas aeruginosa*, and *Enterobacter* sp) and Gram-positive (*Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Bacillus* species) bacteria representing 43.8 % (7/16) and 56.2 % (9/16) respectively of the total isolates (Table 1). Of the Gram-negative isolates, *Pseudomonas aeruginosa* was the commonest (57.1 %; 4/7) whilst *E. coli* was the least isolate (14.3 %; 1/7). *Staphylococcus epidermidis* was the most common Gram-positive isolate (55.6 %; 5/9), whilst *Staphylococcus aureus* (11.1 %; 1/9) was the least. The isolate with the most mean viable count was *Bacillus* sp (11.1×10^6 cfu), while *E. coli* (6.4×10^6 cfu) had the least viable count. On the average, Gram-positive isolates showed the highest mean viable counts compared to Gram-negative isolates (Table 2).

| Bacteria Isolates | SPC (CFU/ml) | Mean CFU/ml | Sample number |
|-----------------------------------|--------------------|--------------------|---------------|
| <i>Escherichia coli</i> | 6.4×10^6 | 6.4×10^6 | 54 |
| <i>Staphylococcus aureus</i> | 9.8×10^6 | 9.8×10^6 | 67 |
| <i>Pseudomonas aeruginosa</i> | | 10.1×10^6 | |
| | 10.2×10^6 | | 12 |
| | 10.4×10^6 | | 32 |
| | 9.8×10^6 | | 59 |
| | 9.9×10^6 | | 71 |
| <i>Staphylococcus epidermidis</i> | | 11.0×10^6 | |
| | 11.2×10^6 | | 16 |
| | 10.8×10^6 | | 26 |
| | 11.4×10^6 | | 41 |
| | 10.8×10^6 | | 78 |
| | 11.0×10^6 | | 84 |
| <i>Bacillus</i> species | | 11.1×10^6 | |
| | 11.2×10^6 | | 8 |
| | 10.8×10^6 | | 23 |
| | 11.4×10^6 | | 91 |
| <i>Enterobacter</i> species | | 7.5×10^6 | |
| | 7.6×10^6 | | 84 |
| | 7.4×10^6 | | 94 |

Table 2: Enumeration of isolates and their mean count (cfu/ml) from the sampled stored blood units.

Discussion

Effective disease prevention and control, healthcare planning, health education, policy decisions, prioritization and execution of healthcare interventions, especially in resource-challenged regions of the world are contingent upon reliable research and baseline information. Unfortunately, in these resource-challenged regions such as Ghana, data are either non-existent or under-sourced. This study investigated bacterial contamination of whole blood at-point-of-transfusion in a tertiary hospital in the western region of Ghana. Previous studies in other regions of Ghana have reported widespread sources of bacterial contamination in whole blood as well as blood components at some major blood transfusion centers including at Accra (37 Military Hospital, Ridge Hospital, National Blood Transfusion Service) in the Greater Accra region [12] and Tamale (Tamale Teaching Hospital) [17] in the northern region. Of note, the previous studies unanimously suggested appraisal of transfusion services across all the ten regions of Ghana to properly assess the quality of blood transfusion services and the associated transfusion-related hazards in order to generate strategies to monitor, supervise and enforce internationally accepted sterile and aseptic procedures. This study estimated bacterial contamination of whole blood at-point-of-transfusion to be 16.5

%). Although, the 16.5 % bacterial contamination detected from 97 blood packs at-point-of-transfusion is lower compared to previous estimates from some blood transfusion centers in Ghana (37 Military hospital [17 %] and Tamale Teaching hospital [17.5 %]) [12, 17]; however, it is higher than those estimated from Accra including Ridge Hospital (12 %) and National Blood Transfusion Service (10 %) [12]. This observation indicates that not much progress has been made with respect to reducing bacterial contamination at transfusion centers since 2009, when major studies were carried out on blood transfusion services, which gave out important recommendations on blood transfusion practices [12,17,20,30]. Comparison of the present finding (16.5 % bacterial contamination of whole blood) with bacterial contamination of whole blood estimated from other Sub-Saharan African countries such as Zimbabwe (3.1 %) [31], Kenya (8.8 %) [1], Nigeria (8.8 %) and Ethiopia (15.33 %) [32], just to mention a sample, clearly show that bacterial contamination of whole blood in Ghana may be common as earlier reported [12,17]. Essentially, the extent of bacterial contamination in Ghana appears more apparent when compared to estimates from developed countries. For example, our estimate is 165 times more than that of France [29], 110 times that of UK [33], and almost 83 times that of USA [34] indicating that blood transfusion services in Ghana urgently needs improvement in order to reduce risk of transfusion-related hazards to prospective blood recipients. The problem of bacterial contamination in Ghana may be the result of lack of standardized screening and testing facilities at some blood banks [10], indiscriminate prescription of blood to patients by clinicians [16], over-concentration on viral blood contaminants to the neglect of bacterial contaminants [35-37] and probably lack of supervision and enforcement of best practices at blood transfusion centers.

Dangers of transfusion-related infections to prospective blood recipients, especially in Sub-Saharan Africa depend not only on the level of blood contamination, but also on the type of pathogenic blood contaminant. For instance, transfusion of a heavily contaminated blood with a non-pathogenic (virulence-free organism) to a recipient may not be as clinically serious as transfusion of blood contaminated sparingly with a high virulent pathogen. Previous studies in Ghana have reported Gram-positive (CoNS, *S. aureus*, *Bacillus sp*) and Gram-negative (*E. coli*, *Yersinia enterocolitica*, *K. pneumoniae*, *P. aeruginosa* [12] bacteriasp, with Gram-positive bacteria been the dominant contaminants [17]. Consistent with previous reports in Ghana [17], other African countries [32,38] and developed countries [39-41], this study identified Gram-positive isolates (56.2 %) as the dominant contaminants of the blood units, with *Bacillus sp* showing the highest growth (11.1×10^6 cfu), whilst Gram-negative isolates (43.8 %) showed comparatively lower growth count, with *E. coli* (6.4×10^6 cfu) showing the lowest growth count.

Identification of strains of isolates as well as their antibiotic resistance patterns is important for prevention and treatment of bacterial infections. This study could not investigate the strains of the bacteria isolates, in order to determine their antibiotic resistance patterns. It is recommended that future study in the study area take a critical look at that. Not with standing, this study provides a baseline information for future investigations in the study area, in view of the fact that this is the first preliminary study at a blood bank in the study area.

Conclusion

Bacterial contamination of at-point-of-transfusion blood at the studied blood bank was 16.5 %, with Gram-positive isolates as the common blood contaminants, suggesting bacterial contamination of stored blood may be common at the study area. Pre-screened and stored blood must be re-screened for bacterial contamination few hours (6-8 h) before transfusion to recipients by using highly sensitive and quick screening methods such as digital counting technology (ProtoCOL3). Also, monitoring, supervision and enforcement of standard sterile and aseptic procedures as well as best practices must be ensured by local hospital-constituted oversight committees and the appropriate authorities (National Blood Transfusion Service, Ghana) at all blood transfusion centers nationwide to save prospective blood recipients from transfusion-related infectious disease complications.

| Bacteria Isolates | Number of Isolates | Common Habitats of Isolates | Gram Reactivity | Frequency* (%) |
|-----------------------------------|--------------------|-----------------------------|-----------------|----------------|
| <i>Escherichia coli</i> | 1 | w, s | - | 6.25 |
| <i>Enterobacter species</i> | 2 | w, s, f | - | 12.50 |
| <i>Bacillus species.</i> | 3 | w, s | + | 18.75 |
| <i>Staphylococcus aureus</i> | 1 | sk, s, n | + | 6.25 |
| <i>Pseudomonas aeruginosa</i> | 4 | w, s | - | 25.00 |
| <i>Staphylococcus epidermidis</i> | 5 | sk | + | 31.25 |
| Total | 16 | | | 100.0 |

Table 1: Bacteria isolates, their habitats, Gram status and frequency of occurrence.

Feces-f, nasal epithelium-n, soil – s, skin – sk, water –w;

*frequency was determined by dividing the number of isolates of each bacteria by the total isolates.

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