

Prevalence of Bacterial Contamination when using a Diversion Pouch during Blood Collection: A Single Center Study in Malaysia

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Abstract

Background: The implementation of diversion pouches is to minimise the risk of bacterial contamination as the initial blood flow is prevented from entering primary bag collections as it is diverted into a pouch. This study was carried out to determine the prevalence of bacterial contamination in the diversion pouches used during blood collections in the Transfusion Department of Hospital Seberang Jaya, Penang, Malaysia.

Methods: BD Bactec™ Fx instrument detection system was performed on 702 samples of 20 mL of diverting blood in diversion pouch. The inoculum volume was 10 mL for both aerobic and anaerobic bottles cultures and incubated for 5 days in the BD Bactec™ Fx instrument. Positive sample was flagged by BD Bactec™ Fx instrument and subculture to identify the species of organism.

Results: The results showed that of 702 samples, 12 (1.7%) were contaminated. The bacterial species identified were coagulase negative *Staphylococcus*, *Staphylococcus aureus* and Gram positive *Bacilli*.

Conclusion: The results strongly suggest that the usage of diversion pouch is of significant importance in reducing bacterial contamination during blood collection.

Keywords: BD Bactec™ Fx, blood collection, blood transfusion, blood contamination, bacterial infections

Introduction

Transfusion transmitted bacterial infection (TTBI) has become a tremendous problem for blood transfusion services in the last few decades. The American Association of Blood Banks (AABB) (2008) (1), reported that the second most common problem faced by blood services in the United States is the bacterial contamination of blood products. The incidence of TTBI in the 1980s was greater than 1 case per 1000 units of blood products. A variety of strategies were developed in the following years in an attempt to decrease the morbidity and mortality risk associated with transfusion associated bacteremia and septic episodes (2–4). The AABB (2008) (1) noted that after the 1990s, tremendous efforts were made to improve the quality of blood to achieve a “zero risk” blood supply.

The majority of bacterial contaminations that contribute to TTBI are from commensal skin microorganisms that are introduced into the blood bag during vein puncture when the blood collection needle enters the skin (5,6). When a needle tip comes in contact with the skin, contaminating bacteria can be detected in the few milliliters of blood passing through the vein

puncture needle (2,7–9). McDonald reported that the first 15 mL aliquot of blood collected has a higher contamination rate than the second aliquot. Thus, excluding the first 20 mL of the initial collection has been suggested to be an effective way of reducing the risks of bacterial contamination in blood products (11), and the use of a diversion pouch during blood collection was recommended (5).

Diversion is based on the principle that the initial flow of blood, which is contaminated by the donor's skin, can be redirected into a pouch in order to reduce the bacterial contamination entering the blood collection bag (10,12). The diversion pouch is attached to the primary collection bag in such a way that the initial aliquot of blood is diverted into the pouch, whereas the remaining flow fills the primary collection bag (2, 15). Results of recent studies support the premise that diversion of the initial 10–30 mL of blood from the main container can reduce the risk of bacterial contamination of the blood product (2, 13,14).

Bruneau et al. reported that the majority of organisms present, in the diverted initial blood flow were skin flora (13). The most common species were Gram-positive cocci (accounting for

81% of the species detected), Gram-positive bacilli (13.9%), and Gram-negative bacilli (5.1%). Use of a diversion pouch can reduce contamination with these bacteria by 40–88 % (10,13,16). Bruneau et al. reported the residual risk of bacterial contamination of whole blood during collection without a diversion pouch to be 2.2% (13). Other studies reported that by diverting the initial 15 ml of whole blood, the residual risk of bacterial contamination was reduced to 0.6% which represents an improvement of 1.6% (17–19).

The use of the diversion pouch as standard operating procedure in Malaysia is not consistent and depends on the resources available. To date, the prevalence of contamination in diversion pouches in Malaysia is unknown, thus it is not clear whether diversion pouches, when used, are a useful safety practice. Therefore, this study was conducted to evaluate the prevalence of bacterial contamination in diversion pouches and to determine if they play a significant role in reducing bacterial contamination during blood collection in Malaysia.

Materials and Methods

Sample collection

This prevalence study was carried out with the binomial categorical outcome variable (i.e., the presence or absence of bacterial contamination in diversion pouches). The study was conducted at Hospital Seberang Jaya and the Microbiology Laboratory, Advanced Medical and Dental Institute (AMDI), Universiti Sains Malaysia for a period of 3 months from March through May 2012. All whole blood donations collected during out-door blood collection drives scheduled by the Blood Bank of Hospital Seberang Jaya were included. The initial 20 mL of blood collected in a diversion bag at the time of blood collection were sent for microbiological analysis. The calculation of sample size was performed using the sample size calculation for estimations, version 1.0.03. (Available at: <http://www.kck.usm.my/ppsg/stats/resources.htm>). In a previous study (20) the proportion of bacterial contamination was reported to be 91%; thus, to achieve an estimated prevalence with a precision of 0.022, a minimal sample size of 634 diversion pouches was required. In this study 702 diversion pouches were obtained during the sampling period.

During the blood collection process, the collection bag was suspended under the donor's arm. A blood pressure cuff or tourniquet was applied to the donor's arm and the site of phlebotomy was disinfected twice with a 70%

isopropyl alcohol swab, which is the standard procedure established by the National Blood Center Kuala Lumpur (21). The diversion pouch was positioned with the notches up and the tube luer adapter assembly down. When the level of blood was approximately in line with the notches, the diversion pouch was full and the remainder of the collection was sent to the main collection bag. The approximate filled volume of the pouch at the notches was 35 mL. Microsoft Excel was used for data entry and analysis.

Detection and isolation of microorganisms at different incubation times

Over the three months study period, 702 blood units were tested by culturing the contents of the diversion pouches using the BD Bactec™ Fx detection system (Becton Dickinson, Franklin Lakes, NJ, USA). Aerobic and anaerobic vials were inoculated with 10 mL of whole blood from each diversion pouch and cultured for 5 days at 37 °C. An aliquot from each vial was removed each day during the 5-day culture period and analysed for the presence/absence of bacteria.

Results

Prevalence of bacterial contamination

Twelve of the 702 diversion pouch samples were positive for bacteria, yielding a prevalence rate of 1.7%. Four of the positive samples contained anaerobic bacteria (coagulase-negative *Staphylococcus*), whereas other eight contained aerobic bacteria (Gram-positive *Bacillus* spp. and *Staphylococcus aureus*) (Table 1).

Detection and isolation of microorganisms at different incubation times

The bacterial species found in the diversion pouch samples presumably came from the donors' skin (Table 1). The presence of bacterial contamination was detectable from day 2 through day 5. *Staphylococcus aureus* and coagulase-negative *Staphylococcus* were detected on days 3 and 4, whereas *Bacillus* spp. were detectable on day 2. These differences could be due to differences in the quantity of the bacterial contamination present in the diversion pouch sample. In most of the contaminated samples, bacteria were detected on the second day of incubation. Figure 1 shows the morphology of the isolated *Bacillus* spp. as visualised by Gram staining. Figure 2 shows the blue-stained diplococcal and tetrads of *S. aureus*; the colonies grown on the subculture plate were large, whitish, and smooth. Figure 3 shows coagulase-

negative *Staphylococcus* as seen under the microscope. Based on the laboratory findings, characteristics of these colonies were similar to those of *S. aureus* and only the biochemical test results differed between the *S. aureus* and coagulase-negative *Staphylococcus* samples.

Discussion

Transmitting infectious agent is a major concern when transfusing blood, and new microorganisms are constantly added to the list of potential causes of transfusion transmitted infection (TTI). Syphilis, a sexually transmitted disease that is caused by *Treponema pallidum*, can be transmitted through blood transfusion if the blood unit was drawn from an infected donor (22,23). Certain viruses including hepatitis B virus, hepatitis C virus, and human immunodeficiency virus, are also recognised causes of TTI (24,25). Appropriate screening of donated blood helps to prevent the spread of these infections through

blood transfusion. Bacterial contamination may lead to a proliferation of organisms and can cause a serious clinical outcome in patients if contaminated blood products are transfused (19). Although the organisms isolated in the present study are generally poor at proliferating at, the recommended storage temperature (1–6 °C), their potential to cause problems should not be underestimated because the ambient temperature in Malaysia is higher.

The commonest bacteria found on human skin are *S. aureus* and coagulase-negative *Staphylococcus*, and they are present on the superficial skin surface. *Bacillus* spp. are transient skin flora and spore formers (17). Being skin commensals, contamination by these organisms is thought to occur primarily during phlebotomy—as a result of improper disinfection and/or skin core removal by the collection needle (3,20). In the present study, four diversion pouch samples were found to be contaminated by *S. aureus*, five were contaminated by coagulase-negative

Table 1: Detection and identification of the bacteria isolated through culture at different incubation times; 702 blood samples were tested

Microorganisms	Time of detection	Parameters	Culture result	Diversion pouch N = 702	% out of 12 positive bacteriological cultures
<i>Bacillus</i> spp.	Day 2	Aerobic cultures	Positive	8	66
<i>Bacillus</i> spp.	Day 2				
<i>Bacillus</i> spp.	Day 5				
<i>Staphylococcus aureus</i>	Day 2				
<i>Staphylococcus aureus</i>	Day 3				
<i>Staphylococcus aureus</i>	Day 5	Anaerobic cultures	Negative	694	33
<i>Staphylococcus aureus</i>	Day 5				
Coagulase-negative <i>Staphylococcus</i>	Day 4				
Coagulase-negative <i>Staphylococcus</i> (anaerobic bottle)	Day 4				
Coagulase-negative <i>Staphylococcus</i> (anaerobic bottle)	Day 4				
Coagulase-negative <i>Staphylococcus</i> (anaerobic bottle)	Day 4	Anaerobic cultures	Negative	698	33
Coagulase-negative <i>Staphylococcus</i> (anaerobic bottle)	Day 4				
Coagulase-negative <i>Staphylococcus</i> (anaerobic bottle)	Day 4				
Coagulase-negative <i>Staphylococcus</i> (anaerobic bottle)	Day 4				
Coagulase-negative <i>Staphylococcus</i> (anaerobic bottle)	Day 4				



Figure 1: *Bacillus* spp. morphology visualised using Gram staining (10× magnification).

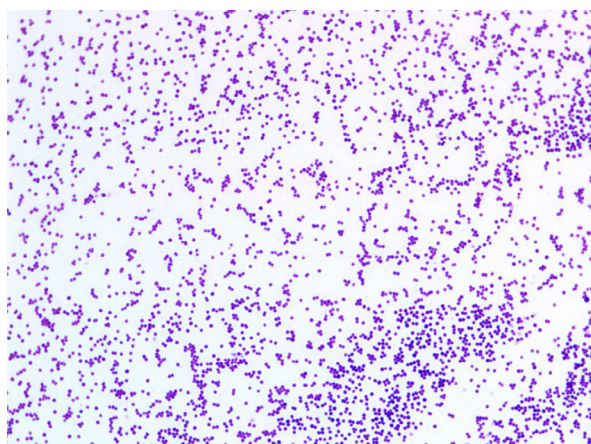


Figure 2: *Staphylococcus aureus* morphology visualised using Gram staining (10× magnification).

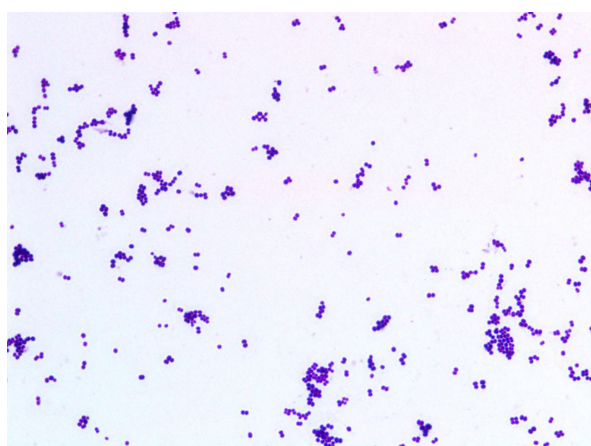


Figure 3: Coagulase-negative *Staphylococcus* morphology visualised using Gram staining (10× magnification).

Staphylococcus, and three were contaminated by *Bacillus* spp. Although not detected in the diversion pouches this study, bacteria such as *Propionibacterium* spp. which colonise the deeper skin layer, sebaceous glands, and hair roots, cannot be eliminated and can still be found in the blood bag (15). The use of a diversion pouch may reduce bacterial contamination, but spore forming bacteria such as *Bacillus* spp. still remain risk for TTBI (17). It is also important to note although contamination of blood by bacteria may be due to improper cleaning of the venipuncture site with disinfectant, it could also be due to a donor having subclinical bacteremia. In this case, diversion of the first few milliliters of blood into a pouch would only reduce the risk of sepsis from skin bacteria (15).

Ramirez-Arcos and Goldman reported that good skin disinfection together with a use of a diversion pouch could reduce bacterial contamination by 77% (26). In the present study, the prevalence of bacteria in the diversion pouches was 1.7%, which is higher than the 0.21% reported by Korte et al. (17) after diversion of 10 mL of blood. A possible explanation for this difference is that the samples collected in the present study were not taken in a controlled environment (i.e., they were collected during out door mobile blood donation drives). The mobile sessions took place in shopping complexes, a school, a mosque, a temple and a factory.

Results of previous studies have indicated that diverting a volume of 20 mL of the initial blood into a diversion pouch is sufficient to eliminate bacterial contamination during blood collection. Approximately 10 studies conducted between 1995 and 2007 tested the effects of diverting different volumes (4). Olthuis et al. (16) reported that diversion of the initial 10 mL of blood during plasma apheresis (4) was enough to reduce bacterial contamination. Wagner et al. (27) demonstrated that diverting 21–42 mL resulted in a reduced load (by about 1 log) of *S. aureus* colonies inoculated on the collection surface (4). In 2007, studies conducted in the Netherlands revealed that the bacterial contamination before diversion (0.9%) decreased by 46% after implementation of the diversion technique (4).

In this study, isopropyl alcohol swabs were used to clean and prepare the venipuncture site. Korte et al. (17) reported that swabbing twice with these swabs reduced the rate of contamination from 0.41% (without swabbing) to 0.25%. McDonald (10) showed that bacterial contamination was significantly reduced when an improved donor arm disinfection technique was

applied together with the use of a diversion pouch (4,10). However, it should be noted that studies of the frequency of bacterial contamination in blood products have limitations including the sensitivity of the culture method and the limited incubation time (19). Thus, the reported prevalence of bacterial contamination is variable and in many cases it is very difficult to compare results among studies due to differences in surveillance and testing methodologies (28). In this study, we used the BD Bactec™ Fx detection system (28) to detect bacterial presence in the diverted blood sample. This widely used standard automated system utilises bacterial production of CO₂ as a marker for bacterial growth (28). The largest possible initial blood sample volume needs to be collected to enhance the probability of detection (10). In this study, 20 mL of the initial blood diverted into the pouch were used for analysis (10 mL for anaerobic and 10 mL for aerobic culture vials).

Screening donors is the first step in maintaining a safe blood supply. Donors with bacteremia have an endogenous source of blood sample contaminant. For example, chronic bacteremia often is observed in patients with syphilis. Blood donated by carriers of *Borrelia burgdorferi*, which is responsible for the tick-derived disease called borreliosis, or *Brucella abortus*, also would pose significant risk to recipients. Bacteremia accompanying alimentary tract infections and alimentary toxicosis, which are caused by bacteria such as *Salmonella*, occurs but is very rare. In order to exclude donors with such illness, it is important to take a comprehensive medical history from each potential donor (29).

Once donors have been screened, the use of diversion pouches is important to help reduce the risk of bacteria entering the primary collection bag. However, the ability of bacteria to proliferate during the storage of blood components is another important factor affecting the blood supply. Red blood cells are stored at 4 °C, which is a temperature that does not permit the proliferation of bacteria (5). After it is processed, plasma is frozen, at -30 °C or lower, which practically eliminates the possibility that bacteria can survive (30). Thus, the risk of bacterial complication is almost nil for plasma or cryoprecipitate transfusion. In contrast, platelets constitute the component that is stored under conditions that promote bacterial proliferation (22 °C). McDonald (10) reported that, platelet suspension in native plasma is an excellent growth medium for bacteria. These organisms

may become opportunistic pathogens and can cause infections in humans if they gain entry into host tissue or blood (3). The level of bacterial contamination at the time of blood collection is generally low, but it can multiply within hours to reach 10⁶ per mL and cause bacteremia if transfused into immunocompromised patients. The adverse effects of receiving a contaminated blood transfusion depend greatly on the bacterial load, the type of bacteria, their pathogenicity, and the underlying clinical condition of the recipient (2).

Data show that the collection of bacteria in diversion pouches is important to help reduce the risk of bacteria entering the primary collection bag. The prevalence of bacterial contamination in diversion pouches may be further reduced by increasing staff diligence in maintaining the standard of care towards donors. (i.e., use of proper disinfection technique and adherence to the standard operating procedure (SOP)). Staff awareness is crucial in adhering to safety measures during blood collection; such measures include using gloves during phlebotomy, changing of gloves immediately if they are torn, punctured, or contaminated, and changing gloves between donors to avoid cross-contamination. Every transfusion center is required to create, maintain and follow a written SOP for every procedure, including donor selection, donor arm preparation and cleansing, proper usage of a diversion pouch, the blood collection process, processing and storage of blood products, and administration of blood to recipients. Strict adherence to SOPs helps reduce bacterial contamination and increases the efficiency of the diversion pouch in trapping any bacterial contamination. For example, the practice of double swabbing with 70% isopropyl alcohol and properly introducing the needle during venipuncture helps reduce contamination by normal skin flora and the skin plug picked up by the needle, thus reducing the bacterial contamination trapped in the diversion pouch (21).

The observed of bacterial rate contamination in the initial diverted blood in our study (1.7%) indicates the need for further improved measures to ensure blood transfusion safety. The most important issues that must be considered when implementing the use of diversion pouches to reduce bacterial contamination are; whether it will be cost effective and whether it will effectively and efficiently decrease bacterial contamination in blood products. This study was limited by the inability to take samples from the primary blood collection bag of the diversion pouches that tested

positive to determine whether the bacteria present in the diversion pouches were also present in the blood bag. In addition follow up of the recipients of blood units for which the diversion pouches tested positive for bacteria was not possible.

Conclusion

In order to reduce bacterial contamination of blood products, prevention based on application of good manufacturing practice is important. One of the best ways to reduce bacterial contamination is the use of a diversion pouch. The majority of bacterial contaminations are derived from normal skin flora or transient skin flora, which are able to proliferate in blood. Using of diversion pouch and following SOPs from blood collection to transfusion minimise the rate of contamination by these microorganisms which in turn improve transfusion safety. The ultimate goal is to achieve zero risk of contamination during blood transfusion.

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Conflict of Interest

None.

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Authors' Contributions

Conception and design, analysis and interpretation of the data: NJ, SRJ, DS

Drafting of the article and collection and assembly of data: NJ, DS

Critical revision of the article for the important intellectual content, final approval of the article and statistical expertise: SRJ, DS

Provision of study materials or patient, obtaining of funding and administrative, technical or logistic support: NJ

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