Impact of Donor Arm Cleaning with Different Aseptic Solutions for Prevention of Contamination in Blood Bags

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Abstract Transfusion associated sepsis cases are encountered occasionally and bacterial transmission remains the major cause. The goal of our study was to compare the efficacy of disinfectants in phlebotomy site preparation. After selection of donor the antecubital fossa area of the arm was disinfected with different types of disinfectants namely spirit (70% isopropyl alcohol), povidone iodine (0.5% w/v available iodine in distilled water), savlon (1.5% v/v chlorhexidine gluconate solution and 3.0% cetrimide solution) and combination of spirit and povidone iodine. Swabs were collected from 20 donors using a sterile forceps, after cleaning with different antiseptic solutions. Swab was streaked on blood agar plate aseptically and the plate was incubated at 37°C for 24 h. Colonies were counted and a single colony was re-cultured by growing on nutrient and Mac-Conkey agar. The biochemical characteristics were determined by performing Gram staining, Motility, Catalase and Oxidase tests. The mean values of colonies were significantly higher with savlon compared to other three solutions. The difference was statistically significant by “*t*” test (*t* values 1.7–3.0; *P* < 0.05). *Staphylococcus epidermidis*, *Staphylococcus* sp., *Streptococcus* sp., *Micrococcus* sp., *Bacillus megaterium* and *Bacillus cereus* were the organisms identified. After completion of bleeding, samples from the bag were aseptically inoculated in aerobic and anaerobic culture bottles to be tested on BacT/Alert system. The bag containing donor’s blood did not show any contamination when three cleanings were carried out using spirit, povidone iodine and spirit respectively.

Keywords Disinfectant · Spirit · Savlon · Povidone iodine · Bacterial contamination · Normal skin flora

Introduction

Donor’s blood is collected in a sterile closed blood bag system to prevent contamination of blood unit. Transfusion associated sepsis cases are encountered occasionally and bacterial transmission remains the major cause of acute morbidity and mortality following post transfusion infection [1–4]. Bacterial contamination of platelet products is generally the most frequent infectious risk associated with transfusion [5–8]. Possible mechanisms of bacterial contamination of blood components include donor bacteremia, contamination during the whole blood collection procedure, contamination of the collection pack or contamination during the blood processing procedure [3, 4, 9]. The types of organisms isolated in these transmissions indicate that the major source of contamination is skin derived [1]. Micro organisms are present on skin of all individuals but are numerous only in relatively moist areas [9].

Literature reports that the normal flora of skin are mainly *Staphylococcus Saprophyticus*, *Micrococcus*, *Diphtheroid*, *Streptococci* and gram negative *Bacilli* [10, 11]. Individuals who receive contaminated blood may develop fever, rigors, skin flushing, abdominal cramps, myalgias, disseminated intra vascular coagulation (DIC), renal failure, cardiovascular collapse and cardiac arrest [12]. The goal of our study is to compare the efficacy of disinfectants in phlebotomy site preparation, as measured by skin surface cultures done before and after antecubital fossa disinfection [12] and check the collected blood bags for contamination.

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Materials and Methods

The study was carried out in the blood bank where 100% blood donations are collected from the voluntary blood donors. The culture work was carried out in a sterile laboratory having GMP standards like air locks, Epoxy flooring, positive pressure, HEPA filters and laminar air flow.

Before starting the project work we compared the swab culture results by obtaining swabs from five donors whose arm was cleaned by sterile cotton swab picked up by gloved hand and by holding it with a sterile forceps. As colony counts were higher when the swab was picked up by hand we decided to use sterile forceps to handle the swabs.

After selection of donor using standard criteria, the donor was requested to lie down on a donor couch and prominent vein in antecubital area was selected. The skin was then disinfected with different types of disinfectants namely povidone iodine (0.5% w/v available iodine in distilled water), savlon (1.5% v/v chlorhexidine gluconate solution and 3.0% cetrimide solution), spirit (70% isopropyl alcohol) and combination of spirit and povidone iodine. Swabs were collected from 20 donors, using each antiseptic solution.

In another experiment swab was collected between each of three cleanings with disinfectants namely spirit, povidone iodine and spirit combination and third cleaning again with spirit.

The swab was immediately kept in sterile test tube after collection. Simultaneously media control, swab control, air control and disinfectant control were also incorporated. Swab was streaked on blood agar plate aseptically and the plate was incubated at 37°C for 24 h. After incubation colonies were counted and a single colony was re-cultured by growing on nutrient and Mac-Conkey agar. The biochemical characteristics were determined by performing Gram staining, Motility, Catalase and Oxidase tests.

After completion of blood donation the whole blood bag was taken to a sterile room and segment was prepared. The sampling site was disinfected with alcohol pad and from the segment 10 ml of blood was collected in sterile

syringe-needle and immediately inoculated 5 ml in each aerobic and anaerobic culture media used in BacT/Alert system (Biomericux Germany). The culture bottles were kept for 3 days in the culture system. The BacT/Alert system and culture bottles provide both a microbial detection system and a culture media with suitable nutritional and environmental conditions for organisms. During incubation the bottles are continuously monitored for the presence of organisms.

Results

This study includes total 88 male and 2 female donors. Age range of these donors was 18–57 years. The experiment using gloved hand picked swab and swab picked up by sterile forceps during the arm cleaning showed 0–12 colonies after arm disinfection using hand picked swab and 0–5 colonies after swab picked up by the sterile forceps. Hence for further experiments swab was picked up by the sterile forceps.

There was no growth on the blood agar plates used for swab control, disinfectant control and medium control. There were five colonies observed on blood agar of area control. Table 1 gives the results of swab culture after using different skin disinfectants. The number of colonies was comparable among different disinfectants before disinfection. The number of colonies after skin disinfection was maximum with savlon (0–167) and minimum with combination of povidone iodine solution and spirit (0–6). The mean values of colonies were significantly higher with savlon compared to other three solutions. The difference was statistically significant by “*t*” test (*t* values 1.7–3.0; $P < 0.05$).

Table 2 shows the data on growth of bacterial colonies, after skin disinfection with spirit, savlon, povidone iodine solution and spirit and povidone iodine combination. Savlon was less effective for skin disinfection while the spirit and povidone iodine combination was the most effective.

Twenty blood donors’ arm skin was disinfected using first spirit, followed by povidone iodine and then spirit.

Table 1 Colony formation after using different skin disinfectants

<i>n</i>	Disinfecting solution	Range of colonies		Mean $\pm$ SD
		Before skin disinfection	After skin disinfection	
20	Povidone iodine solution	11 to >300	0–23	3.3 $\pm$ 6.01
20	Savlon	19 to >300	0–167	35.23 $\pm$ 48.55*
20	Spirit	26 to >300	0–67	8.25 $\pm$ 16.9
20	Combination of Povidone iodine solution and spirit	22 to >300	0–6	0.95 $\pm$ 1.79

n = number tested

* Significantly higher number of colonies after disinfection with Savlon compared to other three disinfectants, by paired “*t*” test ($P < 0.05$)

Table 2 Comparison between different skin disinfectants with respect to growth of bacterial colonies

Bacterial colonies per plate	Spirit		Savlon		Povidone iodine solution		Spirit & povidone iodine combination	
	<i>n</i>	%	<i>n</i>	%	N	%	<i>n</i>	%
0	5	25	2	10	7	35	14	70
1–10	12	60	8	40	11	55	6	30
11–100	3	15	8	40	2	10	0	0
>100	0	0	2	10	0	0	0	0

n = number 20 swabs were tested for each antiseptic or combination of antiseptic solutions

Table 3 Growth of colonies after skin disinfection during three consecutive cleanings

Colonies observed	Before disinfection	Swab culture after			
		First cleaning with spirit	Second cleaning with povidone iodine	Third cleaning with spirit	Blood bag sample culture
Range	22 to >300	0–23	0–8	0–6	0
Mean	–	5.1 ± 6.1	1.85 ± 2.7	0.95 ± 0.79	0

Table 3 gives the results of growth of colonies after skin disinfection during three consecutive cleanings. Number of colonies gradually decreased. The samples collected from the blood bags after completion of donor bleeding did not show any growth. In BacT/Alert system continuously every 10 min the results were recorded for 3 days but no growth was observed in either aerobic or anaerobic culture bottles.

Staphylococcus epidermidis, *Staphylococcus* sp., *Streptococcus* sp., *Micrococcus* sp., *Bacillus megaterium*, and *Bacillus cereus* were isolated from antecubital area of donor arm before donor arm cleaning. After Skin disinfection the organisms detected were *Staphylococcus* sp., *Micrococcus* sp., and *Bacillus megaterium*.

Discussion

Bacterial sepsis is still a significant problem after the transfusion, particularly after the transfusion of platelets. The skin disinfection before venipuncture is crucial in reducing risk of post transfusion infection [13]. Bacterial contamination during whole blood collection occurs due to normal skin flora. There are two types of bacterial flora on human skin, transient and residential. The transient flora is readily removed by washing the arm with soap and water. Hence blood donor is initially instructed to wash his hand adequately [14]. Resident bacteria are securely lodged on and among the cells, cellular debris and lipids. Hence they are difficult to wash and anti-septic solution is necessary for effective cleaning [14]. Dependent on the method and materials used to prepare the donor's arm, bacteria from the surface of the skin can be introduced in the blood collection bag.

There are number of standard disinfection protocols in routine clinical practice. In the transfusion service alcohol and tincture iodine or povidone iodine are often used for donor skin disinfection. Generally tincture iodine is not used for skin disinfection because it causes skin irritation. Povidone iodine solution is preferred because of less skin irritation on skin. In the present study we have evaluated the effectiveness of commonly available disinfectants like spirit, povidone iodine, savlon and combination of spirit and povidone iodine. Care was taken to prevent external contamination by handling the swabs by sterile forceps.

In this study the swabs were cultured using blood agar plate while Nutrient agar and Mac-Conkey agar were used for isolation. Commercially available Tryptic soy agar plates were used by Goldman et al. [12]. They observed zero colonies in 47% donors after cleaning with povidone iodine, 60% after 0.5% chlorhexidine gluconate cleaning and 63% after isopropyl alcohol and tincture Iodine cleaning. In this study the most effective arm cleaning agent was combination of povidone iodine solution and spirit (70% isopropyl alcohol). Savlon was comparatively less effective. The best approach is to scrub the donor's arm thrice with different antiseptic solutions. We observed that the protocol using first spirit, then povidone iodine and last cleaning again with spirit was effective to achieve complete disinfection.

Lee et al. mainly detected *Bacillus* spp. and coagulase-negative *Staphylococcus* on donor arm [13]. In present study, organisms isolated before donor arm cleaning were *S. epidermidis*, *Staphylococcus* sp., *Streptococcus* sp., *Micrococcus* sp., *Bacillus megaterium* and *Bacillus cereus*. After donor arm cleaning *Staphylococcus* sp., *Micrococcus* sp. and *B. megaterium* were detected. Thus even after the

cleaning these three organisms may be present on the skin. All these organisms are normal flora of the skin.

Since the risk of blood product contamination is associated with the presence of bacterial flora on donor arm which can contaminate the whole blood as soon as the blood collection is started, it is advisable to divert first few ml of blood into a separate pouch. McDonald et al. [15] have reported that the effective donor arm disinfection in combination with diversion of initial volume of blood donation from the collection bag is the best approach to prevent blood bag contamination.

In present study, there was no bacterial contamination in the whole blood bag after cleaning with all disinfecting agents. A study published by de Korte et al. [16] reported the prevalence of bacterial contamination in whole blood as 0.35%.

Conclusion

1. Donor arm skin disinfection with combination of 70% isopropyl alcohol (Spirit) and povidone iodine solution is the most effective method compared to a single agent like Spirit, povidone iodine solution or savlon to prevent venipuncture associated contamination in blood products.
2. Savlon as a single disinfectant should not be used for arm cleaning.
3. Antiseptic scrubbing should be carried out thrice using spirit, povidone iodine and spirit or any other aseptic solutions routinely used in the blood bank.
4. Effective donor arm cleaning and use of a closed blood bag system having a pouch to divert initial aliquot of blood are the best strategies to prevent blood product contamination.

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