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Comparison between Chemical and Herbal Disinfectants for Blood Donor Arms

[1,2]Nor Hafeeda Rosdan, [1]Wan Ismahanisa Ismail, [1]Nurdiana Zainuddin, [1]Nor Raihan Mohammad Shabani, [1]Zakaria Ismail, [1]Muhammad Nabil Fikri Roslan, [2]Lim Vuang Hao, [2]Zainoodin Sheik Abdul Kader

[1] *University Teknology Mara Cawangan Pulau Pinang, Campus Bertam. Department of Medical Laboratory Technology, Faculty Health Science, 13200 Kepala Batas, Pulau Pinang, Malaysia.*
[2] *University Science Malaysia, Advance Medical& Dental Institute, 13200 Kepala Batas, Pulau Pinang, Malaysia.*

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ABSTRACT

Background: Skin disinfection is the most crucial part in for blood donor before blood donation. This is because it can lead to bacteria contamination of blood components and cause severe transfusion reactions. The reactions are common to platelets because platelets need to be kept at room temperature. **Objective:** The aim of this study is to determine and compare the effectiveness between chemicals and herb disinfectants for blood donor arms. **Results:** A total 60 subject's surround area of Kepala Batas participates in this study and they were a blood donor. They were divided into 3 group which is group 1 CHG/ lime, group 2 IPA/ lemongrass and group 3 PVI/ betel. Their skin culture samples were collected by direct swabbing techniques using a contact plate for pre-and post-disinfection on both of their arms. Colonies were counted after 24 hours incubation period. Each chemical disinfectants used in this study show significantly different effectiveness ($p > 0.001$) with the percentage of post-disinfection bacteria colony counts reduction for CHG (65%) and PVI (35%) higher than IPA (65%). Each herb used also resulted in significantly different effectiveness ($p>0.001$) with poor post –disinfection bacteria colony count. This was highlighted by the presence of bacterial after post-disinfection. Significantly different effectiveness ($p<0.001$) also resulted when comparing between chemicals and herbs. **Conclusion:** This study showed that chemical disinfectants were effective in reducing bacteria counts but the most effective are CHG and PVI. Introduction of herbs extract as disinfectants also showed effectiveness due to their antibacterial properties and have a potential to be as environmental friendly disinfectants.

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INTRODUCTION

Each year lots of blood have been transfused. Approximately 13, 898, 000 units of red cells or whole blood and 4 million of platelet units are transfused in the United State alone (Breacher and Hay, 2005). Bacterial transmission due to bacterial contaminated blood is not a new problem that has been identified over 60 years ago and it is the commonest cause of complications associated with transfusion. In several countries such as in the USA and United Kingdom, there are reported cases of sepsis due to bacterial contamination attributed to blood component (Pastila *et al*., 2012).

In the USA, from 1986 to 1991 USA Food and Drug Administration (FDA) reported 29 out of 182 (16%) fatalities due to bacterial contamination. Bacterial contamination also had been reported in France between 1994 and 1999 comprised 22% of total transfusion fatalities. The UK Serious Hazards of Transfusion (SHOT) also reported 77% (7/9) fatalities in between 1995 to 2003 also due to bacterial contamination. However, the risk of bacterial infection through bacterial contamination is still the same over many years although the risk of transfusion transmitted disease has been decreasing due to the new testing, methodologies and technologies (Pastila *et al*., 2012). The most frequent bacterial contamination associated with platelet products (Patel *et al*., 2012). Most of the bacteria are unable to proliferate at 0˚C, but able to proliferate in room temperature at which platelets are stored.

Most of recipients that receive transfusion have a very low immune response. Even non-pathogenic bacteria of skin normal flora can lead to fatalities. Mostly the types of bacteria isolated in the several studies are skin derived (Pastila *et al*., 2012). There is an interesting case reported fatal derived from

Corresponding Author: Nor Hafeeda Rosdan, University Teknology Mara Cawangan Pulau Pinang, Campus Bertam. Department of Medical Laboratory Technology, Faculty Health Science, 13200 Kepala Batas, Pulau Pinang, Malaysia.
Tel: +60195499074; E-mail:nhfr.feeda@gmail.com

donor arm probable cause from inadequate donor arm disinfection. After that incident, the donor arm was swab then cultured and the same bacteria isolated. After disinfecting, no bacteria were isolated. This case shows the importance of inadequate donor arm disinfection can lead to bacterial contamination to blood products.

Antisepsis of the donor arm at the antecubital fossa is used to prevent contamination by decreasing the bacterial counts of resident and transient flora (Calfee and Farr, 2002). The study by Calfee and Farr (2002) also reported on 20% of skin bacteria are located in deep layers of the skin or in other structures, so this practice cannot completely prevent contamination. A number of disinfectants have been used to study and compare the effectiveness. Mostly the disinfectants come from chemical based and it is commercialized. There are several contradict between the effectiveness reported in several studies between the commercial antiseptic agents. The most commonly used antiseptic agents are alcohol, povidone iodine, tincture iodine and chlorhexidine (Calfee and Farr, 2002).

For many centuries, people have tried to explore the healing power of different plant extracts and treat diseases (Melendez and Capriles, 2006). Almost 250,000 to 500,000 species found on earth however only 1% has been studied for their pharmaceutical potential (Melendez and Capriles, 2006). According to Chen (2004) the disinfectant that was prepare from plants and the extracts known as herbal disinfectants. Fewer studies have been done on herbal based skin disinfectant. For a many thousands of years, old medicines depend mostly on flower, bark, leaves and fruit of plants.

Recently, a lot of research activities focused on the use of plants as antibacterial, antiviral and so on. Preliminary study was done on the introduction of plant as skin disinfectant using Lime (*Citrus aurantifolia),* Lemongrass *(Cymbopogon citratus)* and betel *(Piper betle)*. The objectives of this research were determining and comparing the effectiveness between chemicals and lime, lemongrass and betel skin disinfectant for blood donor arms.

MATERIALS AND METHODS

A total of 120 random samples were taken using RODAC (Replicate Organism Detection and Counting) contact plates from 60 selected subjects after taking the account of inclusion and exclusion criteria based on donor criteria from the National blood Centre (2008) for this study. PS software Dupont and Plummer (1990) was used to calculate the sample size based on comparing 2 proportions.

The RODAC plates were commercially purchased contain neutralizing agent that able to inactive residual action of disinfectant on the agar plates. Three available disinfectants which are 2% Chlorhexidine gluconate, 70% Isopropyl alcohol, and Povidone Iodine also were commercially purchased. 2% Chlorhexidene gluconate contains active ingredients of 2% Chlorhexidene gluconate with 70% isopropyl alcohol, Povidone iodine contains 2-Pyrrolidinone, 1-ethenyl-, homopolymer, compound with iodine and 70% isopropyl alcohol which only contains a single active ingredient that is 70% isopropyl alcohol. While the herbal disinfectants, lime (*Citrus aurantifolia*), lemongrass (*Cymbopogon citratus*) and betel (*Piper Bete*l) as raw material was purchased from Penang area and the identification was done at Herbarium, USM Penang.

Subjects were divided into three groups for comparison based on the antiseptic skin preparation being tested. Each of 3 groups comprised of 20 subjects. Group 1 was tested with the chlorohexidine gluconate and lime, group 2, isopropyl alcohol and lemongrass and group 3, the providone iodine and betel.

Preparation of lime, lemongrass and betel extract:

The method used for lime extraction was based on Ojiezeh *et al*. (2011) was modified. The lime were chosen, cleansed with distilled water and allowed to dry. The outer part were swabbed with 70% alcohol and allowed to dry before cut open in half and squeezed into sterile container. The lime extraction was allowed to standing for one day and the supernatant was pipette into another sterile container to be kept until used. While the method of extraction for lemongrass and betel was based on Masurkar *et al*. (2011) also was modified. The lemongrass and betel was collected and wash with distilled water. 50 gram of lemongrass and betel were cut into small pieces and dipped into 200 mL distilled water. The mixture was then boiled for 10 to 12 minutes then was filtered using Whatmann filter paper and the solution was kept stored. Sterility test for extracts was done by culturing each solution into Columbia Sheep Blood Agar for 72 hours.

Subject arm disinfection by chemical and herbal disinfectant:

Selected subject was allowed to lie or sit while both of the arms were prepared. Before disinfectant, both arms samples were taken from the subject and labeled as pre- disinfectant and after that 2% Chlorhexidine gluconate was applied to one arm and allowed to dry for 30 second. After 30 second the samples were taken using the RODAC contact plates and labeled as post- disinfectant. This method was repeated with other commercial skin disinfectants at other subjects. The same method was used with the other arm but using the herbal skin disinfectant. After 30 second the samples were taken using the RODAC contact plates and also labeled as post- disinfectant. All plates were incubated at 35 ± 2° Celsius overnight.

Colony count:

Viable counts of organism pre- and post-disinfectant were reported as numbers of colony-forming units (cfu). A viable count is based on the presence of a visible colony develops on the culture medium from each viable unit which may be one organism or a group of many. The presence of greater than 300 colony-forming units on culture plates was reported as Too Numerous To Count (TNTC) rather than the actual number. Difficulty was encountered in accurately calculating individual colonies when greater than 300 colonies were present on any plate.

Microscopic identification of bacterial colonies:

RODAC contact plates of pre- and post-disinfection was randomly selected for each of lime, lemongrass, betel and chemical disinfectants to do a microscopic identification. Microscopic identification of bacterial colonies was done by gram staining certain bacteria colony chosen and observed under microscope.

RESULTS AND DISCUSSION

Bacterial sepsis due to blood contamination especially platelets after transfusion still a significant problem (Patel *et al*., 2012). The skin disinfectant is crucial in reducing the risk to either recipient or blood donor itself. The major aim of this study was to compare and evaluate the effectiveness between lime, lemongrass and betel against chemical skin disinfectants used for blood donor arm since mostly skin preparation prior to phlebotomy predominantly related blood donation.

The effectiveness of skin disinfection has been assessed directly using direct plating technique of skin swabs, where the residual number of bacteria remaining on the skin directly assessed. Malhotra *et al*. (2011) reported on the use of contact plates on the recovery of microorganisms from the test surface have similar efficacy in term of yield and precision with swabbing technique.

All of the methods used for disinfection to disinfect blood donor were same for all chemical disinfection. In this study the techniques utilized by using skin wipe for commercially purchased chemical skin disinfectants were from manufactures. While the all herbal extract was immersed in sterile cotton swab. The time contact for chemical disinfectants and herbal with the skin of blood donor also were monitored to avoid any possibility of error. Poured plate method was used for sterility of extracts by culturing each solution on agar media and the results yield no growth of bacterial after 72 hour of incubation.

Table 1: Demographic data for the distribution of age and gender subjects participate in the study for group 1, group 2 and group 3.

Variable	Category	Group 1 (n= 20)	Group 2 (n=20)	Group 3 (n=20)	Total Group (n=60)
Age	Mean Age	18.65	22.95	19.0	20.20
	Minimum	18	18	19	18
	Maximum	20	30	19	30
Gender	No. of females	11	14	11	36
	No. of Males	9	6	9	24

Table 1 show participants were required to be a minimum of 18 years old and maximum 65 years old. Analysis of variance to compare each group on variable of age indicated that there was a significant difference (F= 18.707; p= < 0.001) for the group (Table 1). A Pearson Chi-Square test indicate that there were no significant differences when comparing the groups (chi square=2.4; p=0. 121) on the variety of gender (Table 1).

Table 2: Mean of bacteria colony counts for pre- and post-disinfection for different types skin disinfectants preparation.

		Pre-disinfection	Post-disinfection
Group 1	2% Chlorhexidine gluconate n=20	116.25	1.15
	Lime extraction n=20	163.10	104.35
Group 2	Povidone Iodine n=20	157.55	22.45
	Lemongrass extraction n=20	157.15	121.70
Group 3	70% Isopropyl alcohol n=20	157.35	1.15
	Betel extraction n=20	188.50	145.55
Total n=120		156.65	66.06

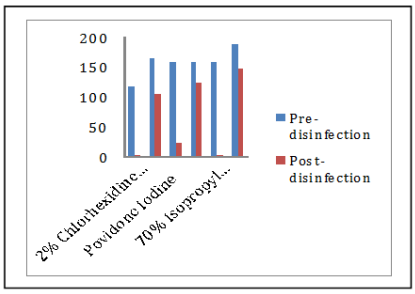

Fig. 1: Mean of bacteria colony counts pre- and post-disinfection for disinfectant.

Table 2 shows chemical disinfectants and herbal results significantly different effectiveness, as measured by the mean of bacteria colony counts on RODAC contact plate cultures for pre- and post-disinfectant. Figure 1 shows clearly on mean of bacterial colony counts after post-disinfection for each group greatly reduce. The greater reduction can be seen in chemical disinfectants more than herbal disinfectants (Fig. 1)

Table 3: The comparative analysis of chemical skin disinfectants 2% chlorhexidine gluconate, povidone iodine, 70% Isopropyl alcohol and their effect on bacterial growth.

Colony count per plate	2% Chlorhexidine gluconate		Povidone Iodine		70% Isopropyl alcohol	
	Pre (%)	Post (%)	Pre (%)	Post (%)	Pre (%)	Post (%)
0	0 (0)	13 (65)	0 (0)	2 (35)	0 (0)	13 (65)
1-10	1 (5)	7 (35)	2 (10)	8 (45)	3 (15)	7 (35)
11-100	9 (45)	0 (0)	8 (40)	3 (10)	7 (35)	0 (0)
100-300	2 (10)	0 (0)	3 (15)	7 (10)	3 (15)	0 (0)
>300	8 (40)	0 (0)	7 (35)	0 (0)	7 (35)	0 (0)
Total	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)

Table 3 shows the comparative analysis on the percentage of pre- and post-bacterial colony count. The percentage of reduction between three different chemical disinfectants almost similar but greater percentages can be seen in skin disinfectant 2% chlorohexidine gluconate and providone iodine (Table 3). Wilcoxon's sign rank test analysis showed the results of pre- and post-disinfection bacterial colony counts significantly difference in effectiveness between three different chemical disinfectants ($Z= -6.729$; $p<0.001$).

This was supported by the study from Goldman *et al*. (1996) they reported the efficacy of chlorohexidine gluconate for donor skin disinfection. Goldman *et al*. (1996) in their study also reported the efficacy of isoprophyl alcohol than providone iodine because the killing of bacteria slower. None show more than 90% reduction. This was highlighted by the absence of counts after disinfection. However, all disinfectant products were effective in decreasing the bacterial contamination of blood donor arm prior to venipuncture.

Table 4: The comparative analysis of herbal skin disinfectants and their effect on bacterial growth.

Colony count per plate	*Lime juice extraction*		*Lemongrass extraction*		*Betel extraction*	
	Pre (%)	Post (%)	Pre (%)	Post (%)	Pre (%)	Post (%)
0	0 (0)	0 (0)	0 (0)	1 (5)	0 (0)	0 (0)
1-10	0 (5)	2 (10)	1 (5)	2 (10)	0 (0)	2 (10)
11-100	9 (45)	11 (55)	8 (40)	9 (45)	7 (35)	8 (40)
100-300	3 (15)	4 (20)	4 (20)	2 (10)	3 (15)	5 (25)
>300	8 (40)	3 (15)	7 (35)	6 (30)	10 (50)	5 (25)
Total	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)

All three herbal disinfectant preparations were also effective in reducing the number of bacteria present on the skin following disinfection but the percentage of bacterial reduction of post-disinfection suggested poor effectiveness (Table 4) when comparing with chemical skin disinfectants that was used in this study. This was highlighted by the present of bacteria counts after disinfection. Statistically significantly different also results in effectiveness pre- and post- disinfection bacterial colony counts between lime, lemongrass ($Z= -5.906$; $p<0.001$).

When comparing the effectiveness between three group, group 1 chlorohexidine gluconate and lime, group 2, isopropyl alcohol and lemongrass and group 3, the providone iodine and betel resulted in significantly different effectiveness, in the respect bacterial count of the reduction ($Z= -8.926$; $p< 0.001$). However, chemical disinfectants were more effective in regard by the absence of bacteria count after disinfection.

As mentions earlier, lot of studies have been done on evaluations of chemical skin disinfectants but less known study have been found using herbal skin antiseptic. In this study, the plants used were extracted using water based and directly used on the skin. The concentration of herbal plants used in respect to its active ingredient was ignored but the effect was shown in the results of bacterial colony counts on skin pre- and post-disinfection. This study has shown that with the used of all these three herbal plants able to reduce the bacterial colony count at post-disinfection but the results was poor when compared to chemical disinfectants that already been established and proven as skin disinfectant used prior to venipuncture.

In this study, we are not trying to prove which antiseptics is the best disinfectant to be used prior to venipuncture. All three show their effectiveness but with significantly different between each other. However, as mention earlier based on the percentage of bacterial reduction chlorohexidine gluconate and providone iodine was more effective than isoprophyl alcohol. Kiyoyama *et al*. (2009) does their study in comparing Isoprophyl alcohol with Isoprophyl alcohol plus Providone Iodine as a skin preparation suggested in the single application of 70% Isoprophyl alcohol enough as optimal antiseptic. His study supported by Goldman *et al*. (1996). Kiyoyama *et al*. (2009) does their study using a lot of blood culture samples to prove their theory while in this study the number of samples is less due to time required to finish this study.

Following microscopic identification using the gram staining method on the bacteria colonies selected, the most common isolated was gram positive organisms. Gram positive organisms were the main microbial flora on the skin.

Conclusion:

The effectiveness of disinfectant used was crucial for blood donation to protect the blood donor and recipient. The blood donor need to be protected because following invasive procedure can introduce organisms to the blood donor and cause diseases or bacteraemia but the most important to the recipient of blood and blood product. Bacteria can be introduced into the blood following donation if skin disinfectant used is not effective or less effective. This can lead to bacterial contamination of blood components. Further study needs to be done in identification of active compounds present in the herbal plants used and concentration required to make all of these plants as skin disinfectants. This just a preliminary study since less study was done on the introduction of herbal plants as skin disinfectant. The main limitation of this study is the small number of subjects plus limited time available.

REFERENCES

Chen., Z.B., 2004. Study And Application Of Herbal Disinfectants In China. Biomedical And Environmental Sciences, 17: 492-498.

Calfee, D.P. and B.M. Farr, 2002. Comparison of Four Antiseptic Preparations for Skin in the prevention of Contamination of Percutaneously Drawn Blood Cultures: a Randomized Trial. Journal of Clinical Microbiology, 40(5): 1660-1665.

De Korte, D., J. Curvers, W.L. de Kort, T. Hoekstra, C.L. van der Poel, E.A. Beckers, J.H. Marcelis, 2006. Effects of skin disinfection method, deviation bag, and bacterial screening on clinical safety of platelet transfusions in the Netherlands. Transfusion, 46(3): 476-485.

Dupont, W.D. and W.D. Plummer, 1990. 'Power and Sample Size Calculations: A Review and Computer Program', Controlled Clinical Trials, 11:116-28.

Goldman, M., G. Roy, N. Frechette, F. Decary, L. Massicotte, G. Delage, 1996. Evaluation of donor skin disinfection methods. Transfusion, 37: 309-312

Kiyoyama, T., Y. Tokuda, S. Shiiki, T. Hachiman, T. Shimasaki, K. Endo, 2009. Isopropyl alcohol compared with isopropyl alcohol plus povidone-iodine as skin preparation for prevention of blood culture contamination. J Clin Microbiol, 47(1): 54-58.

Malhotra, S., A. Dharmadasa, S.M. Yentis, 2011. One vs two applications of chlorhexidine/ethanol for disinfecting the skin: implications for regional anaesthesia. Anaesthesia, 66(7): 574-578.

Mark, E., Brecher and Shauna, N. Hay, 2005. Bacterial contamination of blood components. Clinical Microbiology Reviews, 18(1): 195-204.

Masurkar, S.A., P.R. Chaudhari, V.B. Shidore, S.P. Kamble, 2011. Rapid biosynthesis of Silver Nanoparticles using Cymbopogon Citratus

(Lemongrass) and its Antimicrobial activity. Nano-micro Letter, 3(3): 189-194.

Melendez, P.A. and V.A. Capriles, 2006. Antibacterial propertiesof tropical plants from Puerto Rico. Phytomedicine, 13: 272-276.

National blood Centre, 2008. Transfusion practices guidelines for clinical and laboratory personal. 3rd ed. Malaysia: Ministry of Health, 4-5.

Ojiezeh, T.I., S.E. Nwachukwu and S.J. Udoh, 2011. Antimicrobial effect of citrus aurantifolia juice and veronica amygdalina on common bacteria isolates. Der Pharma Chemica, 3(1): 1-7.

Pastila, S., M. Lonnroth, R. Heikkila, H. Heikkila, P. Carlson, 2012. Bacterial skin flora and contamination of blood components: do we defer blood donors wisely? Vox Sanguinis. 103(2): 93-98.

Patel, Tanvi G. Shukla, V. Rinku, Gupte, C. Snehalata, 2012. Impact of Donor Arm Cleaning with Different Aseptic Solutions for Prevention of Contamination in Blood Bags. Indian Journal of Hematology and Blood Transfusion, 29(1): 17-20.