Evaluation of the Role of Novel Aprotic Dimethyl Sulfoxide in Cutaneous Antisepsis Protocols Prior to Blood Donor Phlebotomy with Isopropyl Alcohol, Povidone Iodine or Chlorhexidine

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Abstract The study was planned to measure the reduction of the load of bacterial flora on the blood donor's arm quantitatively using a three step protocol of donor arm cleansing incorporating either 70% isopropyl alcohol (IPA) or 5% w/v povidone iodine (PVI, 0.5% w/v available iodine) or 4% chlorhexidine gluconate (CHG) with or without the addition of 5% dimethyl sulfoxide (DMSO). Single blind randomized study after obtaining ethical clearance, using the Miles and Misra technique for quantification and matrix assisted laser desorption ionization-mass spectrometry for identification of colony morphotypes on blood donor's skin. The mean pre-cleansing colony forming units (CFUs) was 89,318 and mean post-cleansing CFUs was 132, with a mean reduction of 99.85% with a mean log reduction of 3.24 (95% CI 2.01–4.47) at a *P* value of <0.0001. The post-cleansing CFUs was reduced to zero in all 34 samples in the protocol using CHG with DMSO, in 23 of 31 samples in the protocol using PVI with DMSO and 19 of 29 samples in the protocol using IPA with DMSO. The difference in means of the reduction of CFUs in protocols using CHG with DMSO compared with protocols using PVI or IPA with DMSO and PVI or IPA without DMSO was statistically significant with *P* value of 0.006, 0.0009, 0.015 and 0.05 respectively. The enhanced cutaneous antisepsis effect of

CHG when complimented with DMSO in presence of IPA using the three step protocol of donor arm cleansing could stimulate more research and utilization of this as an additional safety towards the prevention of the problem of bacterial contamination of blood and blood components.

Keywords Isopropyl alcohol · Povidone iodine · Chlorhexidine gluconate · Dimethyl sulfoxide

Introduction

The focus of transfusion services has turned back towards one of the earliest recognised complications of blood transfusion, and bacterial contamination is now again the thrust area after tremendous success in the arena of mitigating the known viral transfusion transmissible infections [1]. It was due to bacterial contamination in glass blood collection bottles that the plastic blood bags saw the dawn of the day [2–4]. Bacterial contamination still remains a concern in platelet transfusion, because platelets storage at ambient room temperature provide the much needed impetus for growth and proliferation of contaminants, which is further catalysed and catapulted by the nutrition from plasma provided for suspension and formulation of the platelet concentrate. The source of platelet contamination is often traced to human skin, being attributed to the skin core that passes into the blood bag during phlebotomy or the persistence of bacteria despite cleansing [5–13]. Overall about 90% of bacterial transmissions due to platelets and 70% due to red cells are attributed to skin derived source [14–16]. Six cases of bacterial infection post blood transfusion were reported by the Serious Hazards of Transfusion Report United Kingdom (SHOT, UK) attributed to skin derived source [17]. Therefore, we

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planned to measure the reduction of the load of bacterial flora on the blood donor's arm quantitatively using either 70% isopropyl alcohol (IPA), 5% w/v povidone iodine (PVI, 0.5% w/v available iodine) or 4% chlorhexidine gluconate (CHG) with or without the addition of 5% dimethyl sulfoxide (DMSO) as step two in the three step donor arm cleansing protocol detailed in “[Materials and Methods](#)”. The other objective was to measure the total bacterial load (resident and the transient flora) and type, and compare it with donor attributes like donor type (donor staying in hospital premises, at risk of having nosocomial transient flora), number of donations (fibrotic dimpling and scarring at previous phlebotomy site scar are known to harbour flora that is difficult to clean and this may be part of the skin core that passes into the blood bag if diversion pouch is not used), work profile of the donor (outdoor vs. indoor work profile) and the period of the year the donation was done (dry hot summer, humid monsoon conditions and winter). To the best of our knowledge after literature search, there is no published research on this topic from India. Therefore, we conducted a pilot study to compare the efficacy of antiseptics protocols using PVI, CHG or IPA with or without DMSO, in cleansing the ante cubital fossa skin prior to phlebotomy. Further find out the association whether determinants like work profile of the donor, previous donation status, prevailing climatic conditions or the stay inside hospital premises have any association with the bacterial load on donor's skin or influence bacterial persistence post cleansing.

Materials and Methods

The study was conducted on blood donors selected for blood donation at the Department of Transfusion Medicine with ethical clearance from the ethics committee of the institute.

Study Design

Single blind randomized study.

Sampling Strategy

Voluntary non-random (convenience sampling), with allocation to antiseptic combination by manual randomization.

Study Methodology

The study tray was made consisting of similar type plastic bottles in which six combinations of 70% IPA, 5% PVI and 4% CHG solution either with or without 5% DMSO. Once

the blood donor consented, a coded chit was randomly asked to be taken out by the paramedic staff posted in the blood donation centre and depending on the code the antiseptic to be used was selected. Sample for the pre cleansing colony forming unit (CFU) enumeration was taken by scrubbing an area of 24 cm² (ensured using bangles made of plastic that were washed with soap and water after each usage, a set of such bangles was procured and rotated for usage) uniformly for about 15 s using pre sterilised swab sticks suspended in sterile, cotton-topped glass tube containing 2 ml of sterile Butterfields phosphate buffer with 0.1% Triton-X-100. Similarly the post cleansing sample was obtained after cleansing of the ante cubital fossa phlebotomy site using the technique as per the departmental standard operating procedure (SOP) of blood donor phlebotomy. The SOP involves first cleansing about 4 by 4 cm area with methylated spirit or IPA by gentle scrubbing, followed by a wait for 30 s and a similar scrub using either PVI followed by a wait for 30 s and a repeat of the first step followed by phlebotomy consequent on the drying up of last step of IPA. The donor arm cleansing was in accordance with the three step technique of the SOP of department, wherein in one study protocol 5% PVI was used, while in other protocols it was substituted with 4% CHG and 70% IPA in the second step only keeping the first and last steps unchanged. Both pre and post cleansing samples were transported to the clinical bacteriology laboratory immediately and processed within 2 h of receipt at the laboratory.

Bacterial Load Quantitation

The tubes containing the swab were vortexed to elute the organisms from the swab into the buffer solution. After squeezing out the swab on the inside of the tube, it was inoculated on a MacConkey agar plate to allow subsequent identification of commensal organism types. For quantitation of viable bacterial load, the Miles and Misra technique was used [18]. Briefly, the solution was logarithmically diluted up to four dilutions in the same Butterfields phosphate buffer without Triton-X-100. Each dilution was inoculated in triplicates of 10 µl each (total 30 µl) on 5% sheep blood agar plates. The dilution at which maximal number of colonies appeared discrete was chosen to count colonies (n). To determine the total number of viable organisms in the total surface area swabbed, the following formula was used:

$$T = (2000/30)n \times 10^x.$$

T = number of organisms in total surface area swabbed, where x is the dilution at which colonies were counted.

Once the colony counts before and after antiseptics was known, the log reduction in organism load was determined using the following formula:

$$\text{Log reduction} = \log_{10}\{T_{\text{pre}}/T_{\text{post}}\}$$

T pre = total number of viable organisms before cleansing and, T post = total number of viable organisms after cleansing.

Identification of colony morphotypes was done using matrix assisted laser desorption ionization-mass spectrometry (MALDI-TOF MS).

Target Preparation

Of the two commonly recommended methods of bacterial whole cell preparation for bacterial species identification using MALDI-TOF MS, the direct colony plating method was used. One colony from an overnight culture of the isolate was spotted onto a 96-well stainless steel target plate (Bruker Daltonics, Bremen, Germany) using a wooden toothpick. Once dry, this was overlaid with 1 µl matrix solution [a saturated solution of α -cyano-4-hydroxycinnamic acid (HCAA) matrix (Sigma-Aldrich) in 50% acetonitrile (Sigma-Aldrich)] and 2.5% trifluoroacetic acid and once again allowed to dry to facilitate crystallisation.

Spectra Acquisition

MALDI-TOF MS was performed on a Bruker Microflex LT bench top instrument controlled by Flex Control software (version 2.0; Bruker Daltonics GmbH, Leipzig, Germany). Spectra were acquired as per the default recommended proprietary settings utilizing the Biotyper pre-processing standard method and the Biotyper Main Spectrum (MSP) identification standard method (linear positive method—20-Hz laser frequency, 20-kV acceleration voltage, 18.5-kV IS2 voltage, 250 ns extraction delay, and 2000–20,000 m/z range). Spectra were recorded in manual and automatic mode. For MALDI Biotyper analyses, calibrated raw spectra were processed with MALDI Biotyper 3.0 (Bruker Daltonics). Default values for bacterial species level identification were used for smoothing (Savitsky–Golay with frame size 25), baseline correction (multipolygon with search window 5 and number of runs 2) normalization (maximum norm) and peak detection (spectra differentiation with signal to noise ratio 3 and threshold 0.001).

Statistical Analysis

Two work sheets were maintained, one for the details of donor variables and the antiseptic code used, other for the microbiology details. After the study was conducted the results were combined in order to maintain blinding. Discrete categorical data was represented in the form of either a number or a percentage (%); proportions were compared using Chi square or Fisher's exact test, depending on their

applicability. All the statistical tests were two-sided and were performed at a significance level of $\alpha = .05$.

Results

The present study enrolled a total of 190 participants on whom the bacterial load was quantified in the pre-cleansing and post-cleansing samples, with the Miles and Misra technique. However six samples were excluded due to sampling problems (sample not labelled clearly as to whether pre or post cleansing) and finally 184 samples were included in the study. The samples from 184 blood donors included 90 donors with outdoor work profile whereas 94 with indoor work profile, and 63 replacement donors who were staying in the hospital premises in order to take care of patient, 114 had come to the hospital only for blood donation. Out of 184, 82 samples were processed during summer months, 56 during monsoon and 46 during winter over 1 year (April 2014–March 2015).

Bacterial identification was carried out in 85 of samples and cultured *Staphylococcus hominis* (74), *Staphylococcus epidermidis* (20), *S. haemolyticus* (7), *Bacillus pumilus* (3), *S. capitis* (2) and *S. nepalensis*, *S. warneri*, *S. cohnii*, *S. xylosus*, *S. caprae*, *S. Saprophyticus*, *S. succinus*, *B. jeotgali*, *B. firmus*, *B. shakeletoni*, *B. subtilis* and *B. megaterium*, *Pseudomonas syringae* and *graminis*, *Acinetobacter schindleri*, *Aerococcus viridians*, *Brevibacillus borstelensis* and *Kocuria palustris* (one each) individually or together.

Table 1 details the comparative mean pre and post-cleansing CFUs, mean percentage reduction and the mean log reduction in the study protocols. The overall mean pre-cleansing CFUs was 89,318 and mean post-cleansing CFUs was 132, with a mean reduction of 99.85% with a mean log reduction of 3.24 (95% CI 2.01–4.47) at $P < 0.0001$.

Post-cleansing, no viable colonies could be counted in 149 samples whereas bacterial persistence was noted in 35 out of 184 samples (19.02%). The CFUs decreased from 75,908 to 180 (99.76%, $P < 0.0001$) in the protocol using PVI, and from 35,537 to 104 (99.70%, $P < 0.0001$) on addition of DMSO to PVI, with log reduction of 3.09 and 3.10 each ($P = 0.6$). The CFUs decreased from 188,656 to 40 (99.97%, $P < 0.0001$) in the protocol using CHG, and from 66,774 to 1 (99.99%, $P < 0.0001$) on addition of DMSO to CHG, with log reduction of 3.57 each ($P = 0.16$). The CFUs decreased from 86,868 to 78 (99.91%, $P < 0.0001$) in the protocol using IPA and from 82,161 to 390 (99.52%, $P < 0.0001$) on addition of DMSO to IPA, with log reduction of 3.25 and 2.80 each ($P = 0.21$).

The post-cleansing CFUs were reduced to zero in all 34 samples in the protocol using CHG with DMSO, in 23 of

Table 1 Bacterial load, persistence, percentage reduction, and log reduction with the different donor arm antiseptics protocols

Protocol (second step antiseptic ^a)	No. of donors	Pre CFU (mean)	Post CFU (mean)	Post CFU 0	Post CFU 1–99	Post CFU > 99	Percent reduction (mean)	Log reduction (mean)	95% CI		Chi square	P value
									Lower limit	Upper limit		
PVI ^a	30	75,908	180	22* (73%)	5	3	99.76	3.09	2.60	3.57	1.01	0.60
PVI + DMSO ^a	31	35,537	104	23* (74%)	3	5	99.70	3.10	2.74	3.46		
CHG ^a	30	188,658	40	27 (90%)	1	2	99.97	3.57	3.06	4.07	3.57	0.16
CHG + DMSO ^a	34	66,774	00	34* (100%)	0	0	100	NA	NA	NA		
IPA ^b	30	86,868	78	24* (80%)	3	3	99.91	3.25	2.82	3.67	3.04	0.21
IPA + DMSO ^b	29	82,161	390	19* (66%)	2	8	99.52	2.80	2.30	3.29		
Overall	184	89,318	132	149 (81%)	14	21	99.85	3.24	2.01	4.47	$P < 0.0001$	

CHG chlorhexidine gluconate, IPA isopropyl alcohol, PVI povidone iodine

* CHG + DMSO versus PVI + DMSO, Chi square = 10.1, $P = 0.0067$

* CHG + DMSO versus IPA + DMSO, Chi square = 13.94, $P = 0.0009$

* CHG + DMSO versus PVI, Chi square = 10.36, $P = 0.0157$

* CHG + DMSO versus IPA, Chi square = 7.5, $P = 0.057$

^a Protocol for antiseptics includes first IPA, then the antiseptic as per the above groups and final IPA scrub again

^b Protocol for antiseptics included all three steps with IPA

31 samples in the protocol using PVI with DMSO and 19 of 29 samples in the protocol using IPA with DMSO. The difference in means of the reduction of CFUs in protocols using CHG with DMSO compared with protocols using PVI or IPA with DMSO and PVI or IPA without DMSO was statistically significant with P value of 0.006, 0.0009, 0.015 and 0.05 respectively as detailed in Table 1.

The data was stratified according to the occupation of the donor, the previous donation status of the participants, on the basis of whether the donor was staying in the hospital premises along with the patient or coming only for blood donation or on the basis of climatic conditions during which the samples were collected the results did not bring out any statistically significant outcome, and the details are depicted in Table 2.

Discussion

The overall bacterial persistence rate in the present study was 19.02% (35 in 184), and the cleaning efficiency was above 99% with all the antiseptics protocols that resulted in statistically significant percentage reduction with $P < 0.0001$. This compares with the results of similar studies [10, 13, 19, 20]. Goldman et al. [13] used a zero post disinfection colony count in at least 70% of the samples for validation of protocol, using the same criteria all protocols in the present study are validated except in the

protocol where DMSO was added to IPA. In the present study antiseptics achieved with protocol using CHG with DMSO was statistically superior compared with the results achieved with protocols using IPA with DMSO ($P = 0.0009$), PVI with DMSO ($P = 0.006$) or PVI without DMSO ($P = 0.01$) or IPV without DMSO ($P = 0.05$). However within the individual protocol the antiseptics achieved did not demonstrate any statistically significant superiority with the addition of DMSO to either PVI ($P = 0.06$) or CHG ($P = 0.16$) or IPA ($P = 0.22$). In context of blood donors; Goldman et al. [13] was the first to report that IPA scrub followed by tincture iodine is more efficacious than PVI (7.5 and 10%), whereas CHG (0.5%) with IPA (70%) to be better than green soap and IPA and McDonald et al. [10] observed tincture iodine (2%) to be superior to PVI (7.5%) and CHG (0.5%). In other clinical settings; some studies report CHG (0.5–2%) and IPA to provide better results than PVI (10%) and IPA (70%), whereas others report equitable efficacy of PVI and CHG [21–25]. whereas Tarrand et al. [26] have shown that DMSO enhances effectiveness of skin antiseptics and reduces contamination rates of blood cultures.

The study has a few limitations as the operator cleansing the donor arm could not be blinded because the colour and consistency of CHG and IPA was different from that of PVI. This may have lead to investigator bias; however the microbiologist was totally blinded. Even though allocation to antiseptic group was randomised, the recruit in the study

Table 2 Stratification of bacterial load, persistence, percentage reduction, and log reduction with determinant variables

	No. of donors	Pre CFU (mean)	Post CFU (mean)	Post CFU 0	Post CFU 1–99	Post CFU > 99	Percent reduction (mean)	Log reduction (mean)	Chi square	P value
Occupation										
Outdoor	90	124,808	173	70	7	13	99.86	3.30	1.65	0.43
Indoor	94	53,882	84	79	7	8	99.84	3.18		
Donation status										
Multiple	114	66,673	106	96	6	12	99.84	3.19	2.76	0.12
First time	70	124,242	163	53	8	9	99.86	3.32		
Hospital stay										
Yes	63	98,027	157	51	2	10	99.83	3.32	4.15	0.12
No	121	93,469	76	98	12	11	99.91	3.23		
Climatic conditions										
Summer (March–June)	82	71,490	163	65	6	11	99.77	3.18	0.87	0.92
Monsoon (July–October)	46	175,787	127	38	3	5	99.92	3.49		
Winter (November–February)	56	41,951	77	46	5	5	99.81	3.11		

was as per the consent obtained from the donor. Enumeration of bacterial load involved dilution of the sample and therefore no viable counts were at the selected dilution and may not actually reflect the status of bacterial load on the donor's skin. The cleansing of superficial layer of skin does not totally eliminate the possibility of bacterial contamination of the product due to the skin core that may pass into the primary blood collection bag because the negative viable colony counts may not actually reflect the antisepsis of the deeper layers of skin [13]. Finally the results of the study do not bring out individually the effects of IPA, PVI or CHG whether with or without DMSO and are actually the results of the three step protocol, wherein IPA is constant in the first and last step and therefore the results reflect the efficacy of the protocol and not individual agent.

Conclusion

There were no residual viable colony counts post cleansing in 100% of samples when DMSO complimented CHG on blood donor's skin prior to phlebotomy following three step protocol utilising IPA in the first step, CHG with DMSO in the second step and IPA again in the third step. The enhanced cutaneous antisepsis effect of CHG when complimented with DMSO in presence of IPA could stimulate more research and utilization of this as an additional safety towards the prevention of the problem of bacterial contamination of blood and blood components.

Compliance with Ethical Standards

Conflict of interest The authors disclose no conflicts of interest and no financial funding from any commercial organisation. The study was funded by PGIMER, Chandigarh, India.

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