Combining cleaning and disinfection – An alternative approach for preventing the contamination of blood products

Abstract

Aim:Disinfection products and protocols have been devised to provide safe blood and blood derivatives for transfusion; however, sepsis is still the leading cause of transfusion reaction fatalities. This raises the question whether disinfection on its own is sufficient for preventing such outcomes and whether cleaning of the arm prior to disinfection can further reduce the amount of skin commensals responsible for the contamination of blood products.
Methodology: One of the blood donor'sarms was disinfected according to the standard protocol and swabs were taken before and after disinfection (Scenario 1). The other arm was cleaned with a hypoallergenic soap-free and alcohol-free wipe and then disinfected (Scenario 2). Swabs from this arm were taken before cleaning, after cleaning and after disinfection. Tryptone soya agar plates were inoculated and incubated at a temperature which facilitates bacterial and fungal growth.
Results:A rate reduction was set and plates that failed this criterion were due to coagulase negative staphylococci.The commonest bacteria identified was *Staphylococcus epidermidis*. Less fungi were isolated on the after-disinfection plates that failed disinfection and these were *Penicillium* sp. and *Cladosporium* sp. Resultant colony counts from both Scenarios were statistically analysed and resulted in a significant reduction of bacterial colony counts post disinfection; however, the after disinfection plates of Scenario 2 had a lower average of colony counts than Scenario 1. Cleaning the skin prior disinfection resulted in a significant reduction of bacterial colony count and leading to a higher average of bacterial reduction in Scenario 2 than Scenario 1. AlthoughScenario 2 resulted in a more successful bacterial reduction, there was no significant difference between the outcomes.
Conclusion: Cleaning the skin prior to disinfection reduces the bacterial load on the skin which makes the disinfection process more effective and reduces the probability of contamination of blood products.

Keywords
Blood Transfusion, blood products, disinfection

Abbreviations
Chlorhexidine (CHX), Tryptone Soya Agar (TSA)

UNDER PEER REVIEW

1. Introduction

The presence of bacteria on the skin is one of the main causes of bacterial contamination of blood products. One of the reasons is inadequate skin disinfection before venepuncture. Bacteria may

remain present on the skin even if skin disinfection is carried out properly. Cleaning of the skin also reduces the bacterial load through the action of detergent monomers. Therefore, to further reduce the bacterial load on the skin, cleaning of the antecubital fossa should be carried out, making skin disinfection more effective. This study is designed to elucidate such statement.

2. Methods

A total of 50 blood donors voluntary participated in this study. The research design of the study may be viewed at Figure 1.

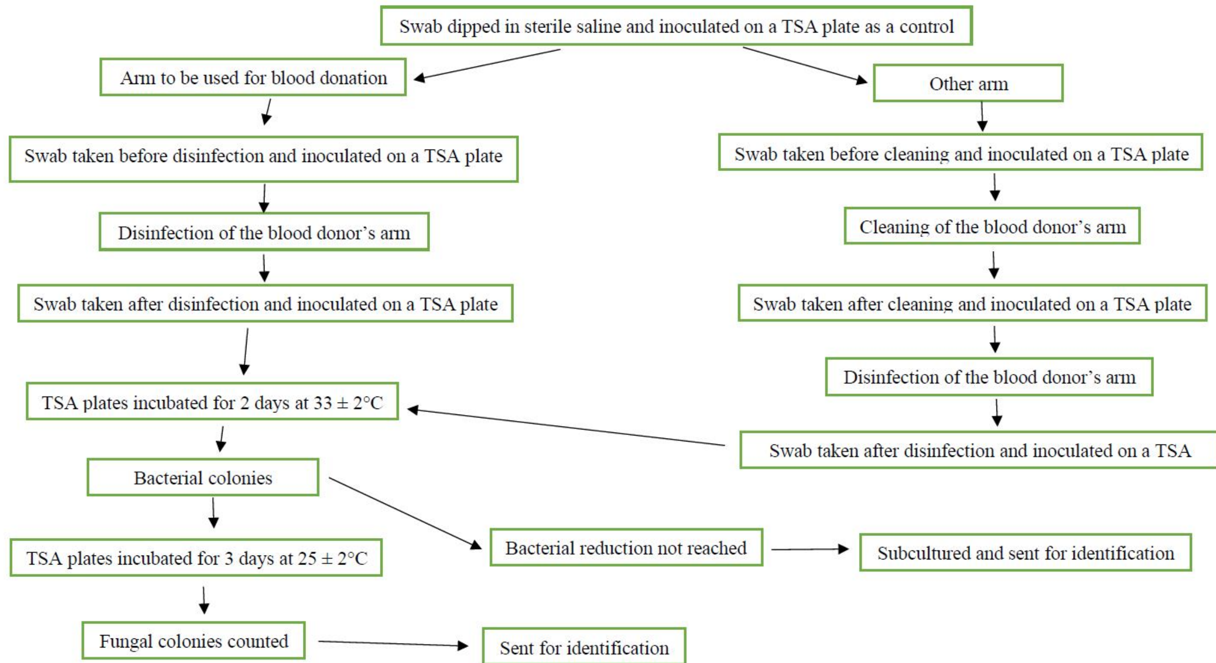

Figure 1: Experimental design.

Since at the time of this study individual saline vials were not available, the same saline bottle was used for all donors. To mitigate this, a quality control step was introduced to make sure that the sterile saline used to wet the sterile swabs was not contaminated between uses. Contaminated saline would haveadded bacteria/fungal colonies to the original number of commensals collected thus jeopardising the reduction rate. So, prior to using the saline for the arm swabbing, a new single wrapped cotton on stick swab was dipped into the bottle and a TSA plate was inoculated by touching the tip of the cotton to the medium. To limit wastage, this TSA plate was divided into 8 segments to accommodate 8 donors.This procedure was performed each time prior the swabbing of a new donor. An assumption was made that if no growth resulted from the saline control, then all bacteria grown on the Tryptone Soya Agar (TSA) which were inoculated from the swabbed arms were indeed skin commensals collected from the donor.

Prior to blood donation one arm of the blood donor was disinfected using alcohol based 0.5% chlorhexidine (CHX)-digluconate solution. Disinfection was performed as follows: A sterile gauze was soaked with a copious amount of disinfectant and then this was rubbed on the antecubital fossa using4 to 6concentric outward circles. The procedure was repeated twice. The antecubital fossa was dried with a clean sterile gauze after which another sterile gauze with0.5% CHX was passed over the site from top to bottom with a single stroke. The area was left to air dry for at least

30 seconds.A TSA plate was used as a saline control for each donor.To determine the baseline for the number of bacteria present, the antecubital fossa was swabbed with a sterile swab saturated with sterile saline (Fresenius Kabi, Cat No.: B230541)prior to disinfection. This swab was used to inoculate a TSA(BioMérieux, Cat No.: 43711) plate. This swabbing and inoculation procedure was repeated post disinfection of the arm. To compare the efficacy of cleaning prior to the disinfection, the other arm of the same donor was cleaned using a generic hypoallergenic soap and alcohol-free wipe.Cleaning was performed using 4 to 6concentric outward circles as previously described.The arm was swabbed before the cleaning step, after the cleaning step and after the disinfection. The same disinfection protocol was used.

The TSA plates were then incubated in an incubator set at a controlled temperature (Poleko-Aparatura, Poland) of 30°C-35°C for 2 days. On the 2nd day bacterial colonies were counted using a colony counter (Bel-Art® SP Scienceware®, USA). TSA plates that contained more than 300 bacterial colonies were marked as '>300'.Once bacterial colony counts were taken, the plates were transferred in another incubator (Helmer, Inc, USA) set at a temperature 25°C ± 2°C for 3 days. This transfer facilitated fungal growth. On day 5, fungal colonies were counted, and the total number of colonies were noted down on the same results sheet. Quality control plates were also checked in parallel with the other plates and if any, the total number of colonies were noted down.

Bacterial and fungal counts from the before and after plates were statistically compared to determine if cleaning the donors' arm prior to disinfection was effective in reducing the bacterial load and thereby preventing contamination of blood products.

The Blood Establishment where this study was performed has a bacterial reduction target of 92.5%. The formula to calculate reduction was taken from Debrincat, Gialanze et al. (2021) and can be viewed at Figure 2.

$$100 - \left[\left(\frac{\text{Bacterial colonies after disinfection}}{\text{Bacterial colonies before disinfection}} \right) \times 100 \right]$$

Figure 2: Bacterial reduction calculation (adopted from Debrincat 2021).

Post-disinfection TSA plates which did not reach the bacterial reduction target had each colony with a different morphologic characteristic subcultured on aTSA plate to obtain purity plates which were then sent to an external laboratory for identification to identify the bacteria which showed resistance to the disinfection process.

2.1. Statistical analysis

UNDER PEER REVIEW

Statistical analysis was carried out using Statistical Package for Social Sciences software version 28 (IBM, USA). The frequency distribution of the variables within this dataset was checked using the Shapiro-Wilk and Kolmogorov-Smirnov tests. The variables included in the normality tests were the bacterial and fungal counts obtained from the TSA cultures of the 1st and 2nd Scenarios, as well as the bacterial reduction rates of both Scenarios. The Shapiro-Wilk and Kolmogorov-Smirnov tests were also used to determine whether parametric or non-parametric tests should be used during further statistical analysis of the mentioned variables. The variables were determined to be non-normally distributed; therefore, non-parametric tests were used to analyse the variables in this dataset. The bacterial and fungal counts obtained from Scenario 1 and the bacterial and fungal counts from Scenario 2 are groups of data that are related, in this case by a single donor, for each set of 5 swab samples. The Wilcoxon Signed Ranks Test is the non-parametric test which was used to determine if there is a significant difference between the number of bacterial and fungal colonies counted in Scenario 1 (before and after disinfection). The same statistical test was used to determine if there are any positive and negative differences between the groups of data in Scenario 2. The Friedman test is another non-parametric test that is used to determine if there is a significant difference between 3 or more dependant. In this case, it was used to determine if there is a significant difference between the number of bacterial and fungal colonies counted in Scenario 2 (before cleaning, after cleaning and after disinfection). Once the statistical difference is calculated, a p-value is given which determines the significance of the differences between the variables in each Scenario. The pairwise comparison test was used to determine if there is a significant difference between different groups of data of bacterial colony counts from the same Scenario and different Scenarios. The Friedman test was used to determine if there is a significant difference between different groups of data of fungal colony counts in both Scenarios. Next, the Binomial test was used to determine whether the average bacterial reduction rate of both Scenarios reached the desired 92.5% target. Frequency tables were used to analyse the outcomes (either pass or fail) of each Scenario using values of the bacterial reduction rate. Finally, the Chi-Square test was used to determine if there is a significant difference between the outcomes of both Scenarios.

3. Results

A total of 50 blood donors participated in this study. Samples which resulted in a higher bacterial count in the post-cleaning TSA plates than in the pre-cleaning TSA plates were excluded from this study. A higher bacterial count in the post-cleaning TSA plates indicates that contamination took place during arm swabbing or inoculation of the swabs on the TSA culture media. For this reason, samples from 3 blood donors were excluded. Therefore, samples from 47 blood donors will be included in the data analysis of this study. Furthermore, the maximum number of bacterial colonies counted per plate was set at 300. All control swabs were negative confirming that no contamination was due to the saline being used.

3.1. Bacterial Counts

As can be seen from Table 1, for Scenario 1, that is where the disinfection only was employed, the before disinfection plate yielded an average of 225 bacterial colonies while the after-disinfection plate yielded an average of 4 bacterial colonies. The maximum number of bacterial colonies in the before disinfection plate was 300 colonies (which was the maximum number of colonies to be counted). After disinfection was carried out, the maximum number of bacterial colonies was 43 colonies. The minimum number of bacterial colonies in the before disinfection plate was 8 colonies. After disinfection was carried out, the minimum number of bacterial colonies was 0 colonies.Table 1, also depicts Scenario 2 where cleaning of the arm occurred before the disinfection, shows that

UNDER PEER REVIEW

the before cleaning plate yielded the most bacterial colonies, with an average of 230 colonies. After cleaning, the average bacterial colonies were 161 colonies. The average number of bacterial colonies yielded on the after-disinfection plates was 3 colonies. The maximum number of bacterial colonies in the before cleaning plates was 300 colonies. Similarly, the maximum number of bacterial colonies in the after-cleaning plates was 300 colonies. After disinfection, the maximum number of bacterial colonies was 19 colonies. The minimum numbers of bacterial colonies in the before cleaning plate was 11 colonies. The minimumnumber of bacterial colonies in the after-cleaning plates was 5 colonies. After disinfection, the minimum number of bacterial colonies was 0 colonies.

Descriptive Statistics (Bacterial counts)

		N	Range	Minimum	Maximum	Mean
Scenario 1	Before Disinfection - Day 5	47	292	8	300	224.6
	After Disinfection - Day 5 (D2)	47	43	0	43	4.17
Scenario 2	Before Cleaning - Day 5	47	289	11	300	23.49
	After Cleaning - Day 5	47	295	5	300	161.06
	After Disinfection - Day 5 (C3)	47	19	0	19	2.68

Table 1: Analysis of bacterial counts

The average number of bacterial colonies in the after-cleaning plate from Scenario 2 is less than the average number of bacterial colonies in the before disinfection plate from Scenario 1. Furthermore, the average number of bacterial colonies in the after-disinfection plate from Scenario 2 is lower than the average number of bacterial colonies in the after-disinfection plate from Scenario 1. Figure 3 is an example of a set of TSA plates used for Scenario 1 (A and B) and Scenario 2 (C-E) for sample number 25 which shows the effect of cleaning and disinfection on the number of bacterial colonies. TSA plates form Scenario 1 show a near total reduction of bacterial colonies. The after-cleaning TSA plate from Scenario 2 shows a total reduction in the after-disinfection TSA plate.

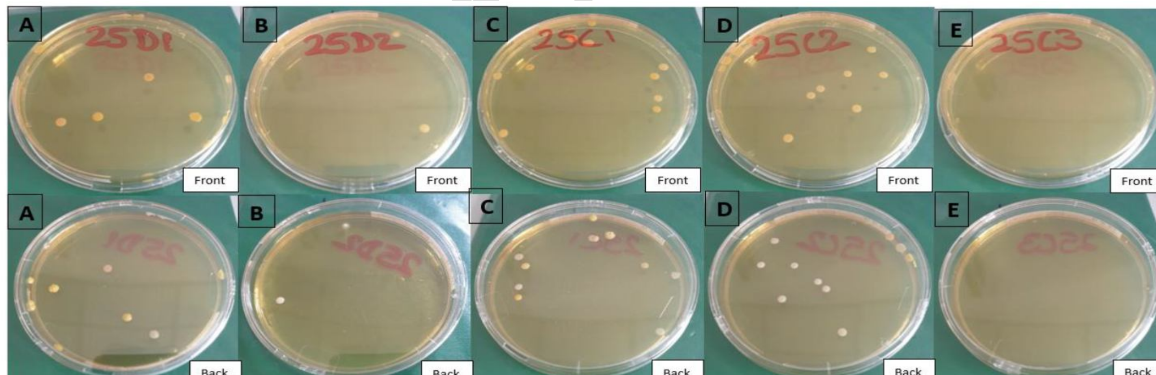

Figure 3: Bacterial colonies on TSA plates from Scenario 1 (A-B) and Scenario 2 (C-E).
The 'before disinfection' plate (A) of Scenario 1 shows 8 bacterial colonies while the 'after disinfection' plate (B) shows one bacterial colony. The 'before cleaning' plate (C) of Scenario 2 shows 11 bacterial colonies, the 'after cleaning' plate (D) shows 10 bacterial colonies and the 'after disinfection' plate (E) shows no visible growth. These plates are as observed on day 2 of incubation.

3.2. Fungal Counts

As can be seen in Table 2, for Scenario 1, the 'before disinfection' plate yielded the most fungal colonies, with an average of 0.35 colonies, while the after-disinfection plate yielded an average of 0.02 fungal colonies. The maximum number of fungal colonies in the before disinfection plate was 15 colonies while the maximum number of fungal colonies in the after-disinfection plate was 1 colony. Meanwhile, the minimum number of fungal colonies in the before disinfection plate and in the after-disinfection plate was 0 colonies. The same table shows that in Scenario 2, the 'before cleaning' plate yielded the most fungal colonies, with an average of 0.15 colonies. while the after-cleaning plate yielded an average of 0.06 fungal colonies. The maximum numbers of fungal colonies in the before cleaning, after cleaning plates and the after-disinfection plate were 6 colonies, 2 colonies and 1 colony, respectively. Meanwhile, the minimum number of fungal colonies in the before cleaning, after cleaning and after disinfection plates was 0 colonies.

Descriptive Statistics (Fungal counts)

		N	Range	Minimum	Maximum	Mean
Scenario 1	Before Disinfection - Day 5	47	15	0	15	0.34
	After Disinfection - Day 5 (D2)	47	1	0	1	0.02
Scenario 2	Before Cleaning - Day 5	47	6	0	6	0.15
	After Cleaning - Day 5	47	2	0	2	0.06
	After Disinfection - Day 5 (C3)	47	1	0	1	0.02

Table 2: Analysis of Fungal counts

The average number of fungal colonies in the 'after cleaning' plate from Scenario 2 is less than the average number of fungal colonies in the 'before disinfection' plate from Scenario 1. The after-disinfection plates from Scenario 1 and Scenario 2 had the same average number of fungal colonies. Figure 4 is an example of a set of TSA plates used for Scenario 1 (A and B) and Scenario 2 (C-E) for sample number 43 which shows the effect of cleaning and disinfection on the number of fungal colonies. TSA plates form Scenario 1 show a total reduction in the number of fungal colonies post-disinfection. A total reduction of fungal colonies was observed from the before cleaning plate to the after-cleaning plate in Scenario 2. The after-disinfection plate from Scenario 2 did not yield any fungal growth.

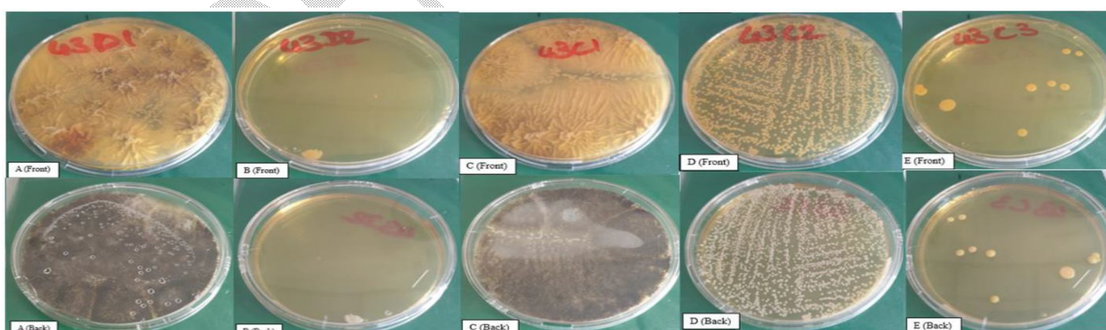

Figure 4: Fungal colonies on TSA plates from Scenario 1 (A-B) and Scenario 2 (C-E).
The 'before disinfection' plate (A) shows fungal growth which covers almost all the agar while the 'after disinfection' plate (B) shows no fungal growth. The 'before cleaning' plate (C) shows 6 fungal colonies. The 'after cleaning' plate (D) shows confluent bacterial growth across the agar but no fungal growth and the 'after disinfection' plate (E) shows 10 bacterial colonies but no fungal growth. These plates are as observed on day 5 of incubation.

3.3. Identification of bacteria and fungi

Bacterial colonies in post-disinfection TSA plates from both Scenarios that did not reach the bacterial reduction target (92.5%) were subcultured and sent for identification. All fungal colonies on the post-disinfection TSA plates had their mother plates sent for identification. The most common bacteria identified were the Gram-positive Staphylococci. These include *Staphylococcus epidermidis*, *Staphylococcus hominis*, *Staphylococcus haemolyticus*, *Staphylococcus pasteuri*, *Staphylococcus saprophyticus* and *Staphylococcus capitis.* Other less commonly identified genera of bacteria were *Kocuria palustris* and M*icrococcus luteus.* The following bacteria were only identified in the after-disinfection plates of Scenario 1: *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus capitis* and *Staphylococcus pasteuri.* Bacteria which were only found in the after-disinfection plates of Scenario 2 were *Staphylococcus hominis*, *Staphylococcus saprophyticus*, *Kocuria palustris and Micrococcus* luteus*.* The white colony in plate number D32-2 could not be identified; however, microscopy showed that these are palisading Gram-positive rods. All identification results had
a confidence interval of 99.9% except for one. Identification results of the purple colony from plate number C38-3 had a 50% confidence level for *Enhydrobacteraerosaccus*and a 50% confidence interval for *Moraxella osloensis.* The identified bacteria can be found in Table 3.

Plate number	Confidence Interval	Bacterium identified
13C-3	99.90%	*Staphylococcus hominis*
13C-3	99.90%	*Staphylococcus hominis*
13D-2	99.90%	*Staphylococcus haemolyticus*
13D-2	99.90%	*Staphylococcus pasteuri*
13D-2	99.90%	*Staphylococcus epidermidis*
16C-3	99.90%	*Staphylococcus saprophyticus*
16D-2	99.90%	*Kocuria palustris*
25D-2	99.90%	*Staphylococcus capitis*
32D-2	99.90%	*Staphylococcus capitis*
32D-2	N/A	Not identified
38C-3	50.00%	*Enhydrobacteraerosaccus / Moraxella osloensis*
38C-3	99.90%	*Micrococcus luteus*
39C-3	99.90%	*Staphylococcus saprophyticus*
39C-3	99.90%	*Kocuria palustris*
50D-2	99.90%	*Staphylococcus epidermidis*
50D-2	99.90%	*Staphylococcus epidermidis*

Table 3: The identified bacteria

Most of the identified bacteria have a 99.9% confidence interval. However, one bacterial colony has a 50.0% confidence interval, and another bacterial colony does not have a confidence interval since it could not be identified.

Only 2 fungi were identified, and these were *Penicillium* sp. and *Cladosporium* sp. The*Penicillium* sp. was found in the after-disinfection plate 25 of Scenario 1 while the*Cladosporium* sp. was found in the after disinfection plate 38 of Scenario 2.

3.4. Data analysis of Scenario 1

UNDER REVIEW

The Wilcoxon test was used to determine if there is a significant difference between the mean colony counts on the before disinfection plates and the mean colony counts on the after-disinfection plates. The null hypothesis specifies that there is no significant difference between the before and after disinfection plates. The null hypothesis is accepted if the p-value is larger than 0.05. The alternative hypothesis specifies that there is no significant difference between the before and after disinfection plates.

For the bacterial colony counts (Table 4 A), the p-value is less than 0.05; therefore, the alternative hypothesis is accepted. There is a significant difference of bacterial colonies between the before disinfection and the after-disinfection plates of Scenario1.As for fungal colonies (Table 4B), the p-value obtained is 0.414 which is more than 0.05; therefore, the null hypothesis is accepted. This means that there is no significant difference of fungal colonies between the before disinfection and the after-disinfection plates.

Related-Samples Wilcoxon Signed Rank Test Summary (A)	
Total N	47
Test Statistic	0
Standard Error	94.23
Standardized Test Statistic	-5.986
Asymptotic Sig. (2-sided test)	<.001

Related-Samples Wilcoxon Signed Rank Test Summary (A)	
Total N	47
Test Statistic	1.500
Standard Error	1.837
Standardized Test Statistic	-0.816
Asymptotic Sig. (2-sided test)	0.414

Table 4: The result of the Wilcoxon test for bacterial (A) and Fungal (B) colony counts from Scenario 1.

Most of the TSA plates before disinfection contained 300 colonies or more. Figure 5 shows that 31 TSA plates resulted in a reduction of 300 bacterial colonies or more after disinfection; 7 TSA plates resulted in a reduction ranging from 0 - 50 bacterial colonies and 4 TSA plates resulted in a reduction ranging from 100-150 bacterial colonies; 3 TSA plates resulted in a reduction ranging from 50 – 100; 1 TSA plate resulted in a reduction ranging from 150-200 and 1 TSA plate resulted in a reduction ranging from 200 - 250 bacterial colonies. Only negative differences can be observed in Figure 7, which means that every before disinfection plate resulted in less bacterial colonies in the after-disinfection plate. The number of ties is 0 which means that there were no after disinfection plates that remained with the same number of bacterial colonies.

UNDER PEER REVIEW

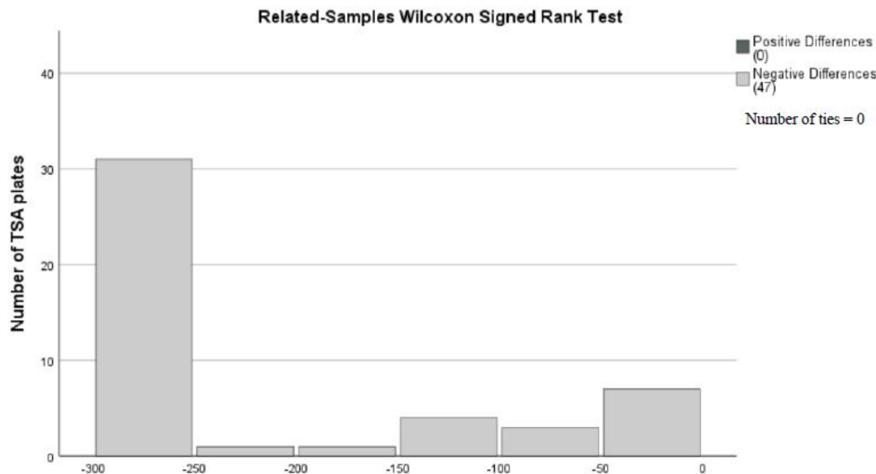

Figure 5: The difference in frequency of bacteria colony counts between the before disinfection plates and the after-disinfection plates of Scenario 1.

More than 30 plates resulted in a reduction of over 300 bacterial colonies in the after-disinfection plates. The absence of positive differences means that there were no after disinfection plates that contained more bacterial colonies than the before disinfection plates.

Most of the TSA plates before disinfection contained no fungal colonies. Before disinfection, 2 TSA plates contained fungal growth. Sample 40 yielded 15 fungal colonies and sample 16 yielded 1 fungal colony. Only 1 TSA plate contained fungal growth after disinfection. Sample 24 yielded 1 fungal colony after disinfection. As can be observed in Figure 6, 2 TSA plates resulted in negative differences ranging from -10 to -15 fungal colonies and only 1 resulted in a positive difference ranging from 0 to 5 fungal colonies. This means that 2 TSA plates resulted in a decrease of fungal colonies from the before disinfection plates to the after-disinfection plates. Only one TSA plate resulted in more fungal colonies in the after-disinfection plate. The number of ties is 44, which means that there were 44 TSA plates which remained with the same number of fungal colonies in the after-disinfection plates. In fact, most TSA plates had 0 fungal colonies in the before disinfection and after disinfection plates.

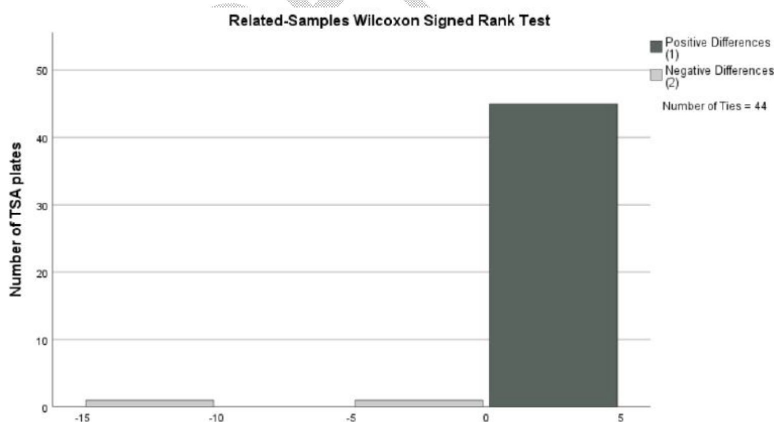

Figure 6: The difference of fungal colony counts between the before disinfection plates and the after-disinfection plates.

1 TSA plate had a positive difference while 2 TSA plates had a negative difference. Most of the TSA plates resulted in no difference. This shows that most before disinfection plates contained the same fungal colony counts as in the after-disinfection plates.

3.5. Data analysis ofScenario 2

The number of bacterial colonies before cleaning resulted as follows: 30 TSA plates contained 300 or more bacteria colonies; 5 TSA plates contained between 0 – 49 bacterial colonies; 4 TSA plates contained between 50 – 99 bacterial colonies; 4 TSA plates contained between 100 – 149 bacterial colonies; 2 TSA plates contained between 150 – 199 bacterial colonies and 2 TSA plates contained between 200 – 249 bacterial colonies. The number of bacterial colonies after cleaning. 20 TSA plates contained 300 or more bacteria colonies; 14 TSA plates contained between 0 – 49 bacterial colonies; 8 TSA plates contained between 50 – 99 bacterial colonies, and 4 TSA plates contained between 100 – 149 bacterial colonies. Most TSA plates contained from 0 to 4 bacterial colonies after disinfection, 5 TSA plates contained bacterial colonies ranging between 1-10 and 2 TSA plates contained bacterial colonies ranging between 40-45.As can be seen in Figure 7, 26 TSA plates resulted in negative differences while no TSA plates resulted in positive differences. This means that 26 TSA plates resulted in a decrease of bacterial colonies from the before cleaning plates to the after-cleaning plates. No TSA plates resulted in an increase of bacterial colonies in the after-cleaning plates. Since the maximum number of bacterial colonies counted per plate was set at 300, the number of ties is 21. This means that there were 21 TSA plates which remained with the same number of bacterial colonies in the after-cleaning plates.

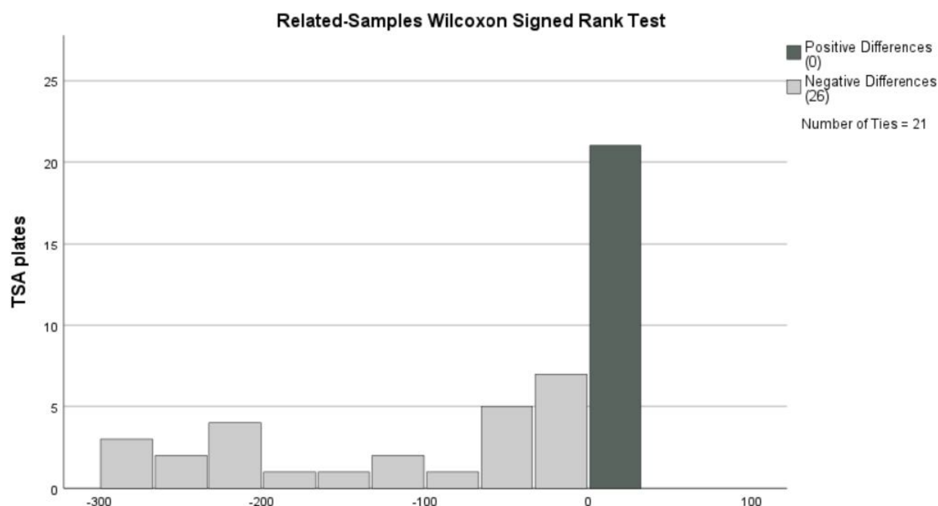

Figure 7: The differences in bacteria colony counts from the before cleaning plates to the after-cleaning plates.

26 plates resulted in a reduction of bacterial colonies in the after-cleaning plates. The absence of positive differences means that there were no after cleaning plates that contained more bacterial colonies than the before cleaning plates. The number of ties are automatically included as positive differences even though the number of positive differences is 0.

Figure 8 shows that 47 TSA plates resulted in negative differences which means that there was a reduction of bacterial colonies from the after-cleaning plates to the after-disinfection plates. No TSA plates resulted in positive differences which means that no TSA plates resulted in an increase of bacterial colonies from the after-cleaning plates to the after-disinfection plates. Thenumber of ties is 0, which means that there were no TSA plates which remained with the same number of bacterial colonies in the after-disinfection plates.

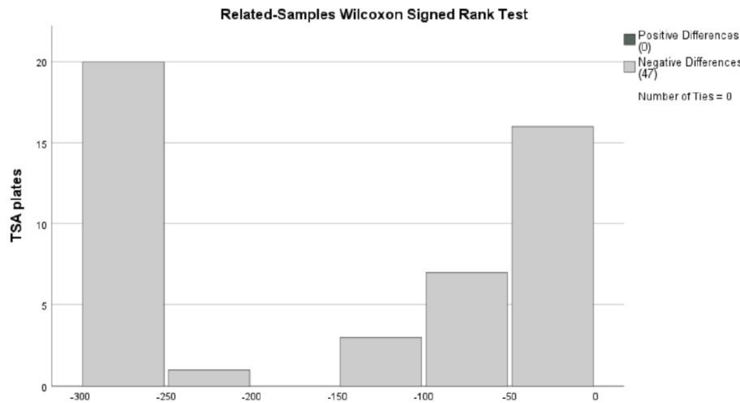

Figure 8: The negative difference in bacterial colony counts from the after-cleaning plates to the after-disinfection plates.

All TSA plates resulted in a reduction of bacterial colonies in the after-cleaning plates. The absence of positive differences means that there were no after disinfection plates that contained more bacterial colonies than the after-cleaning plates.

Fungal colony counts obtained in Scenario 2resulted in 45 TSA plates in the before cleaning plates contained no fungal colonies. Only 2 TSA plates contained fungal growth before cleaning; 1 before cleaning plate contained 1 fungal colony and another before cleaning plate contained 6 fungal colonies. After cleaning, 45 TSA plates contained no fungal colonies; 1 after cleaning plate contained 1 fungal colony and 1 after cleaning plate contained 2 fungal colonies. After disinfection, 46 TSA plates contained no fungal colonies and only 1 TSA plate contained 1 fungal colony.As can be observed in Figure 9, 1 TSA plates resulted in a negative difference ranging from -4 to -6 fungal colonies and 1 TSA plate resulted in a positive difference ranging from 2 to 4 fungal colonies. This means that 1 TSA plate resulted in a decrease of fungal colonies from the before cleaning plates to the after-cleaning plates. Only one TSA plate resulted in more fungal colonies in the after-cleaning plate. The number of ties is 45, which means that there were 45 TSA plates which remained with the same number of fungal colonies in the after-cleaning plates. In fact, most TSA plates had 0 fungal colonies in the before cleaning and after cleaning plates.

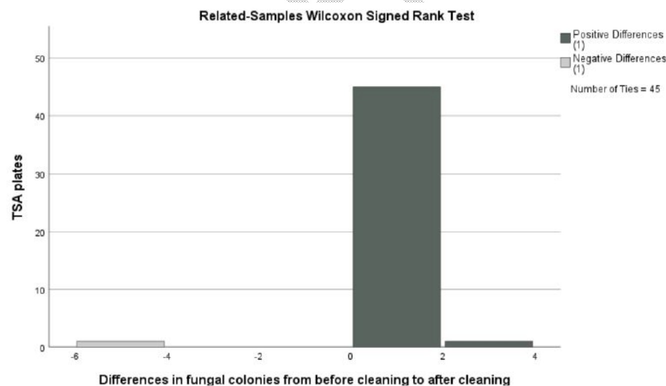

Figure 9: The difference of fungal colony counts between the before cleaning plates and the after-cleaning plates.

1 TSA plate had a positive difference, and 1 TSA plate had a negative difference. Most TSA plates showed no difference in fungal colonies in the after-cleaning plates since most of them contained 0 fungal colonies. The number of ties is automatically included as positive differences even though the number of positive differences is 0.

CPD PREVIEW

Figure 10 shows that 2 TSA plates resulted in negative differences ranging from 0 to -2 fungal colonies and only 1 resulted in a positive difference ranging from 1 – 2 fungal colonies. This means that 2 TSA plates resulted in a decrease of fungal colonies from the after-cleaning plates to the after-disinfection plates. Only one TSA plate resulted in more fungal colonies in the after-disinfection plate. The number of ties is 44, which means that there were 44 TSA plates which remained with the same number of fungal colonies in the after-disinfection plates. In fact, most TSA plates had 0 fungal colonies in the after cleaning and after disinfection plates.

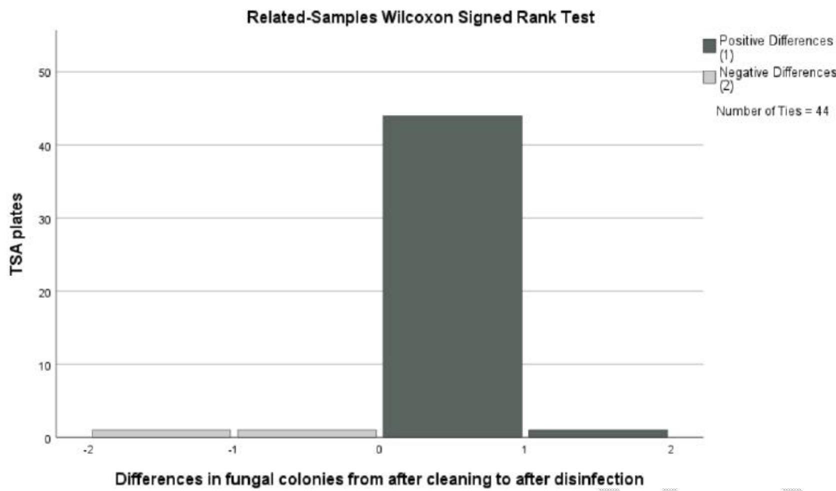

Figure 10: The difference of fungal colony counts between the after-cleaning plates and the after-disinfection plates.
1 TSA plate had a positive difference, and 2 TSA plates had a negative difference. Most TSA plates showed no difference in fungal colonies in the after-disinfection plates.

The Friedman test was used to compare the mean number of bacterial and fungal colonies between groups of data in Scenario 2. For the bacterial counts, the null hypothesis specifies that there is no significant difference between the before cleaning, after cleaning and after disinfection plates. The null hypothesis is accepted if the p-value is larger than 0.05. The alternative hypothesis specifies that there is no significant difference between the before cleaning, after cleaning and after disinfection plates. Table 5 shows that the p-value is less than 0.05; therefore, the null hypothesis is rejected. This means that there is a significant difference between the mean bacterial colony counts in the before cleaning, after cleaning and after disinfection plates in Scenario 2. For the fungal counts the null hypothesis specifies that there is no significant difference between the before cleaning, after cleaning and after disinfection plates. The null hypothesis is accepted if the p-value is larger than 0.05. The alternative hypothesis specifies that there is a significant difference between the before cleaning, after cleaning and after disinfection plates. Table 5 shows that the p-value is larger than 0.05; therefore, the null hypothesis is accepted. This means that there is no significant difference of the mean fungal colony counts between the before cleaning, after cleaning and after disinfection plates in Scenario 2.

Test Statistics Bacterial Counts (A)	
Total N	47
Chi-Square	87.461
df	2
Asymp. Sig.	<.001

Test Statistics Fungal Counts (B)	
Total N	47
Chi-Square	.500
df	2
Asymp. Sig.	.779

Table 5: Friedman test for bacterial (A) and fungal (B) colony counts in Scenario 2.

For the bacterial counts (A), the p-value indicates that there is a significant difference of bacterial colony counts between the groups of data in Scenario 2. For fungal counts (B) The p-value indicates that there is no significant difference of fungal colony counts between the groups of data in Scenario 2.

3.6. Comparing the colony counts from both Scenarios

The pairwise comparison test was used to compare different groups of data from the same Scenario or different Scenarios. The null hypothesis specifies that there is no significant difference between groups of data if the p-value is larger than 0.05. According to the results obtained in Table 6, there is no significant difference of bacterial colony counts between the after-disinfection plates of Scenario 1 and Scenario 2, since the p-value is larger than 0.05 (p-value = 0.794). However, the p-value of the before cleaning and after cleaning plates of Scenario 2 is smaller than 0.05 which means that there is a significant difference between the 2 groups of data. This shows that there was a significant reduction of bacterial colony counts after cleaning the antecubital fossa of the blood donor. Furthermore, the before disinfection and after disinfection plates of Scenario 1 and the before cleaning and after disinfection of Scenario 2 had a p-value smaller than 0.05 which means that both Scenarios resulted in a significant reduction of bacterial colonies. This also applies for the before disinfection plates in Scenario 1 and the after-cleaning plates in Scenario 2, which means that there is a significant difference between the 2 groups of data. This shows that the bacterial load on the antecubital fossa is lower after cleaning than without.

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.
After Disinfection (Scenario 1) – After Disinfection (Scenario 2)	.085	.326	.261	.794
After Cleaning (Scenario 2) -Before Disinfection (Scenario 1)	-.702	.326	-2.153	.031
After Cleaning (Scenario 2) -Before Cleaning (Scenario 2)	.766	.326	2.348	.019
Before Disinfection (Scenario 1) – AfterDisinfection (Scenario 1)	2.755	.326	8.448	<.001
Before Cleaning (Scenario 2) – After Disinfection (Scenario 2)	2.734	.326	8.382	<.001

Table 6: Pairwise comparison test for bacterial colony counts.

The p-value indicates that there is no significant difference between the after-disinfection plates of both Scenarios. However, the p-values indicate that there is a significant difference between the before disinfection plates and after disinfection plates of Scenario 1 and between the before cleaning plates and the after-disinfection plates of Scenario 2. There is also a significant difference between the after-cleaning plates of Scenario 2 and the before disinfection plates of Scenario 1 and between the after cleaning plates and the before cleaning plates of Scenario 2.

UNDER PEER REVIEW

The Friedman test was used to compare the mean number of fungal colonies between different groups of data in both Scenarios. The null hypothesis specifies that there is no significant difference between the different groups of data in both Scenarios. The null hypothesis is accepted if the p-value is larger than 0.05. The alternative hypothesis specifies that there is no significant difference between the different groups of data in both Scenarios. Table 7 shows that the p-value is larger than 0.05 ($p = 0.924$); therefore, the null hypothesis is accepted. This means that there is no significant difference of fungal colony counts between the different groups in both Scenarios.

Test Statistics	
Total N	47
Chi-Square	.901
df	4
Asymp. Sig.	.924

Table 7: Friedman test for fungal colony counts in both Scenarios.
The p-value indicates that there is no significant difference of fungal colony counts between thegroups of data in both scenarios.

3.7. Determining the average bacterial reduction of both Scenarios

The average bacterial reduction rate of both Scenarios was calculated using the binomial test. The null hypothesis specifies that the mean bacterial reduction is 92.5% and is accepted if the p-value exceeds the 0.05 level of significance. The alternative hypothesis specifies that the mean bacterial reduction differs significantly from 92.5% and is accepted if the p-value is smaller than the 0.05 criterion. Table 8 shows that the p-values of the bacterial reduction targets of both Scenarios are less than 0.05 ($p = <0.001$). Thus, the null hypothesis is rejected, which means that the bacterial reduction of both Scenarios is significantly different than 92.5%. Table 9 also shows the minimum bacterial reduction reached in both Scenarios. The minimum bacterial reduction of Scenario 1 is 77.8% while that of Scenario 2 is 85.9%.

		Category	Observed Prop	Exact Sig. (2-tailed)
Scenario 2 – Bacterial reduction	Group 1	<= 92.5	.06	<.001
	Group 2	>92.5	.94	
	Total		1.00	
Scenario 1 – Bacterial reduction	Group 1	<= 92.5	.13	<.001
	Group 2	>92.5	.87	
	Total		1.00	

Table 8: The binomial test used for the determination of the average bacterial reduction of both Scenarios.
Bacterial reductions of both Scenarios are significantly different than 92.5% cut off.

Descriptive Statistics	N	Mean	St. Deviation	Minimum	Maximum
Bacterial Reduction (Scenario 1)	47	97.474	5.3760	77.8	100.0
Bacterial Reduction (Scenario 2)	47	98.255	3.2121	85.9	100.0

Table 9: The average bacterial reduction of both Scenarios.
Scenario 2 has a higher bacterial reduction than Scenario 1.

UNDER PEER REVIEW

3.8. Determining the most successful Scenario

A bacterial reduction with a value less than 92.5% was considered as a 'fail' while a bacterial reduction with a value of 92.5% or higher was considered as a 'success'. Table 10 (A) shows that in Scenario 1, 41 disinfection plates resulted in a successful bacterial reduction with a value of 92.5% or higher. Only 6 after disinfection plates resulted in a failed bacterial reduction. From Table 10 (B)one may see that in Scenario 2, 44 disinfection plates resulted in a successful bacterial reduction with a value of 92.5% or higher. Only 3 after disinfection plates resulted in a failed bacterial reduction. From the results obtained it can be observed that there are more successful bacterial reductions in Scenario 2 than in Scenario 1.

Scenario 1 (A)			
		Frequency	Percentage
Valid	Fail	6	12.8
	Success	41	87.2

Scenario 2 (B)			
		Frequency	Percentage
Valid	Fail	3	6.4
	Success	44	93.6

Table 10: Outcome of Scenario 1 (A) and Scenario 2 (B).

The Chi-Square test was used to compare the outcomes of both Scenarios and to determine which Scenario was more successful. The null hypothesis specifies that there is no significant difference between the outcomes of Scenario 1 and Scenario 2. It is accepted if the p-value exceeds the 0.05 level of significance. The alternative hypothesis specifies that there is a significant difference between the outcomes of Scenario 1 and Scenario 2. It is accepted if the p-value is less than the 0.05 criterion. Table 11shows that the percentage of successful bacterial reduction outcome in Scenario 2 (93.6%) exceeds by 6.4% the percentage of successful bacterial reduction outcome in Scenario 1. However, Table 12 shows that this percentage difference is not significant since the p-value (0.293) exceeds the 0.05 level of significance.

			Scenario		
			1	2	Total
Bacterial reduction outcome	Fail	Count	6	3	9
		Percentage	12.8%	6.4%	9.6%
	Success	Count	41	44	85
		Percentage	87.2%	93.6%	90.4%
Total		Count	47	47	94
		Percentage	100.0%	100.0%	100.0%

Table11: A crosstabulation showing the percentages of the outcomes of both Scenarios.
Scenario 2 has a higher percentage of successful bacterial reduction than Scenario 1.

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	1.106	1	.293

Table 12: Chi-Square test.
This table shows that the percentage difference of the outcomes of both Scenarios is not significant.

4. Discussion

This study evaluated the efficacy of disinfection and cleaning of the blood donors' arms by carrying out statistical analysis on 2 Scenarios: Scenario 1 involved the swabbing of the blood donors' arms before and after disinfection while Scenario 2 involved the swabbing of the blood donors' arms

UNDER PEER REVIEW

before cleaning, after cleaning and after disinfection. It is important to note that whenever Scenario 1 and Scenario 2 are mentioned, they are referring to this study.

Arghittu et al.(1)stated that the lower the bacterial load, the more effective the disinfection process. The average number of bacterial and fungal colonies was highest in the before disinfection plates of Scenario 1 and in the before cleaning plates of Scenario 2 while the after-disinfection plates of both Scenarios contained the least average number of bacterial and fungal colonies. This can also be observed in a study conducted byTan et al.(2). In such study, participants were split into 2 groups where disinfection was carried out using either 70% IPA or 0.9% normal saline. Irrespective of the assigned group, one of the participant's arm was disinfected with a circular motion while the other arm was disinfected with a back and forth scrubbing motion. Each arm was swabbed with 3M Clean-Tarce to measure the amount of microorganisms' adenosine triphosphate (ATP) in reactive light units (RLU) present on the antecubital fossa before and after each technique. The results of Tan et al.(2) are in agreement with the findings from Scenario 1 and Scenario 2 i.e., the median RLU was highest before disinfection and lowest after disinfection.

The results from the present study show that the mean number of bacterial colonies in the after-disinfection plates of Scenario 2 was lower than the after disinfection plates of Scenario 1. Furthermore, the mean number of bacterial colonies in the after-cleaning plates of Scenario 2 was lower than the mean number of bacterial colonies in the before disinfection plates of Scenario 1. Cleaning also reduces fungal growth as seen by the reduction in the mean number of fungal colonies from the before cleaning to the after-cleaning plates. However, both Scenarios resulted in the same mean number of fungal colonies in the after-disinfection plates. Patelet al.(3)demonstrated the efficacy of different disinfectant solutions by comparing the number of bacterial colonies post disinfection. The disinfectants used in the study by Patel et al. were spirit, savlon, P-I solution and a combination of P-I solution and spirit. Comparing both studies shows that the mean number of bacterial colonies post disinfection in Scenario 1 was lower than the mean number of bacterial colonies obtained after disinfection with savlon and spirit. On the other hand, disinfecting the skin with P-I and a combination of P-I solution and spirit resulted in a lower mean number of bacterial colonies than Scenario 1.

In this study bacterial colony counts resulted in only negative differences in both Scenarios while fungal colony counts resulted in both negative and positive differences. The reason for positive differences of fungal colony counts could be due to swabbing of an area of the skin which was not disinfected and/or cleaned or due to improper cleaning and/ordisinfection techniques. Since bacteria on the skin are much more abundant than fungi, the TSA plates showed more bacteria colonies than fungal colonies. In fact, most before disinfection plates in Scenario 1 contained no fungal growth. Consequently, the disinfection process in Scenario 1 did not result in a significant reduction in fungal colonies. This shows that the current disinfection protocol does not result in a significant reduction of fungal colony counts. There was not a significant difference in fungal colony counts between both Scenarios. This means that a cleaning step before disinfection might not result in a significant reduction of fungal colony counts. This is attributed to the fact that most TSA plates in both Scenarios contained no fungal growth. Contradicting this, is a study conducted by Fu et al.(4)which tested the efficacy of different hand hygiene products on pig skin. The study showed that there was a slightly higher log reduction of fungal colonies on pig skin when it was scrubbed with Walch liquid soap for 30 seconds followed by 15 seconds of contact time with Jifro disinfectant gel than if the skin was only disinfected for 15 seconds of contact time(4). In the current investigation,

UNDER PEER REVIEW

there was a significant reduction of bacterial colony counts in Scenario 1. In other words, the current disinfection protocol at the hosting blood establishment results in a significant reduction of bacterial colony counts on the antecubital fossa of the blood donor prior to blood donation. In addition,there was a significant reduction between the before disinfection plates and after disinfection plates of Scenario 1 and between the before cleaning and after disinfection plates of Scenario 2. This shows that both Scenarios are effective in reducing the bacterial load on the skin. However, there was no significant difference between the after-disinfection plates of both Scenarios. Furthermore, the same table shows that there was a significant difference between the before cleaning and after cleaning plates of Scenario 2 and between the after-cleaning plates of Scenario 2 and before disinfection plates of Scenario 1. This shows that although the number of bacterial colony counts in the afterdisinfection plates of both Scenarios was not significantly different, cleaning the antecubital fossa prior to disinfection significantly reduced the bacterial load on the skin.

Rönneret al. (5)carried out a study to compare two skin cleaning procedures: soap and water and a no-rinse cleanser. The inside of each participants' lower arms was contaminated with a filter paper dipped in a bacterial solution. One arm was cleaned with a wash glove dipped in soap and water in circular motions while the other arm was cleaned with a wash glove dipped in a no-rinse cleanser. Both arms were rinsed and dried and a contact agar plate was used to measure the number of bacteria on the skin. LikeScenario 2 of the current investigation, both skin cleaning procedures resulted in a significant reduction of skin commensals. However, the number of the remaining skin commensals were higher after cleaning the skin with the no-rinse cleanser(5). This evidence supports the theory that if a cleaning step was introduced, disinfection would be more effective. In fact, cleaning the skin before disinfection resulted in a significant reduction of bacterial colonies and TSA plates resulted in a higher bacterial reduction. On the other hand, the findings of the study carried out by Mihalacheet al. (6)were not in agreement with the findings of the current investigation. They showed that cleaning the skin with a wet wipe does not result in a significant reduction in organic dirt and bacteria from the skin. Participants in the study contaminated their hands with a lactic fermented liquid containing a non-pathogenic bacterium (*Lactobacillus delbrueckii*ssp. *Delbrueckii*). They were then instructed to perform 5 hand cleaning proceduresusing warm water with bland soap followed by drying the hands with a paper towel, cold water and bland soap followed by drying the hands with a paper towel, cold water followed by drying the hands with a paper towel, wet wipe, and antibacterial wet wipe. To release the dirt after each hand cleaning procedure, the participants dipped their hand in a beaker with sterile water and clenched andreleased their fist for 20 seconds. The amount of dirt was measured with a bioluminescence test which allows for the measurement of ATP in liquids. Cleaning the skin with warm water and soap was the most effective hand cleaning procedure in eliminating bacteria and organic dirt from the skin while cleaning the skin with a wet wipe was the least effective method(6).

Currently, the bacterial reduction target of the Blood Establishment where the present study was performed is of 92.5% and both Scenarios of the current investigation exceeded it. Results have shown that the average bacterial reduction of both scenarios is significantly higher than 92.5%. Scenario 1 has an average bacterial reduction of 97.5% while Scenario 2 had a higher bacterial reduction, with an average of 98.3%. Therefore, it is more likely that cleaning before disinfection results in a higher bacterial reduction, which is in accordance with the results of the other studies(5,7,8). A higher bacterial reduction lowers risk of contaminated blood products; thus, it lowers the risk of haemolytic transfusion reactions post transfusion.

UNDER PEER REVIEW

Celere et al. (7)assessed the efficacy of 2 disinfection techniques: 10% P-I and 0.5% CHX. Results showed that the average bacterial reduction following the application of 10% P-I was 98.57% in blood agar media and 98.87% in mannitol salt agar media. Both average bacterial reductions are higher than the bacterial reduction obtained in Scenario 1. The reason for this could be due to the two-step technique of 10% P-I, which involved antibacterial 10% P-I followed by alcoholic 10% P-I. The same study obtained lower average bacterial reductions following the application of 0.5% CHX which were an average of 94.38% bacterial reduction in blood agar media and an average of 95.06% in mannitol salt agar media(7). These average bacterial reductions are lower than the average bacterial reduction obtained in Scenario 1. Another study carried out by Jensen et al. (8)showed that the average bacterial reductions following the cleaning of the skin with bland soap or antibacterial soap containing 1% chloroxylenol was higher than the average bacterial reductionof Scenario 2. Cleaning the skin with bland soap resulted in an average of 99.49% bacterial reduction while cleaning the skin with an antibacterial soap resulted in an average of 99.87% bacterial reduction. It was hypothesised that the high average bacterial reductions were due to the mechanical removal of bacteria during scrubbing(8). The study conducted by(5), had similar findings. It showed that cleaning the skin with a wash glove dipped in soap and water or in a no-rinse cleanser resulted between 4-5 log reduction of skin commensals, which is equivalent to 99.99% - 99.999% bacterial reduction. The protocol used in the current investigation was based on the study carried out by Debrincat et al. (9), at the same establishment. Like the current investigation, the antecubital fossa of participants in said study was swabbed to obtain the number of bacteria before disinfection. This was followed by disinfecting the same area with 0.5% CHX and resulted in a bacterial reduction of 98.4%, which is higher than the bacterial reduction obtained in the current investigation. The bacterial reduction of Scenario 2 from the current investigation was lower by 0.1%.

The chi-square test showed that there was no significant difference between the outcomes of both Scenarios. The study conducted byTan et al (2) showed that there is no significant difference between the outcomes of skin disinfection with 70% isopropyl alcohol and skin cleansing with 0.9% normal saline. Likewise, the study of Basavarajegowda et al.(10) resulted in an insignificant difference between the outcomes of the arm washing group and the non-arm washing group. The arm of participants in both groups was disinfected with antibacterial 10% P-I in a horizontal and vertical movements followed by alcoholic 10% P-I with an outward circular movement(10). In the present studyScenario 2 resulted in more successful bacterial reductions i.e., more TSA plates reached the 92.5% bacteria reduction target than the TSA plates in Scenario 1. The failure rate of Scenario 1 was 12.8%. This could be due to the small sample size of this study, which only included 47 valid blood donors. A small variation would negatively skew the results. The study carried out by Debrincatet al.(9)showed that the success rate of the current disinfection protocol was 92.3%, which is higher than the success rate obtained in Scenario 1 of this study. The reason for this could be due to a larger sample size in said study. Since the current study was carried out during the summer months, it could also be due to an increased bacterial load on the antecubital fossa which would result in a less effective disinfection process. However, the climate change between seasons is not extreme. The success rate of Scenario 2 was 93.6%, which is higher than the success rate of the study of Debrincatet al.(9).

Out of the 47 TSA plates, 9 of them failed disinfection i.e., they did not reach the 92.5% reduction target. The most common micro-organisms cultivated werecoagulase negative staphylococci (11). In

UNDER PEER REVIEW

fact, most TSA plates that did not reach the reduction target were all identified as staphylococci whichwere coagulase negative. The 2 colonies on 50D-2 and 1 of the colonies on 13D-2 were identified as *Staphylococcus epidermidis*, which is one of the most abundant skin flora(12).

*Staphylococcus epidermidis*protects the skin from colonisation of *Staphylococcus aureus* by degrading the proteins required for biofilm formation(11). It also contains genes which help protect it from harsh environments on the skin, for example high salt concentrations(13). This makes it more likely to contaminate blood products and cause bacterial transmitted infections. *Staphylococcus epidermidis* grows at a slow rate in platelet concentrates when compared to other bacteria, which may be undetectable by culture methods during the early stages of contamination(14). Additionally, *Staphylococcus epidermidis* can form biofilms (15). Bacteria that can form biofilms express stress phenotypes such as efflux pumps which remove antibiotics. This makes *Staphylococcus epidermidis* resistant to antibiotics. Efflux pumps also eliminate disinfectants(16). In fact, *Staphylococcus epidermidis* was only isolated in two after disinfection plates of Scenario 1 but not in the after-disinfection plates of Scenario 2. This could imply that cleaning is effective in eliminating those bacteria which are resistant to alcohol-based disinfectants due to the friction applied on the skin.

Staphylococcus capitis was identified on another two TSA plates that failed disinfection. It is mostly commonly found on the skin of the head and neck(17). *Staphylococcus capitis* is one of the commonest isolated bacteria in contaminated platelet units and it has been the causative agent of sepsis on multiple occasions(18). A study conducted by Tran et al.(19)showed that *Staphylococcus capitis* was totally eradicated when using chlorhexidine gluconate as a disinfectant; however there was a 29% regrowth when using IPA alone. *Staphylococcus capitis* can be difficult to detect if traditional methods are used since it has a tendency to form biofilms, leaving only a few bacteria in their planktonic state(18). Furthermore, *Staphylococcus capitis* is 64 times more resistant to disinfectants when it is in its biofilm state rather than when it is in its planktonic state(20). Like *Staphylococcusepidermidis*,*Staphylococcus capitis* was isolated on two after disinfection plates of Scenario 1 but not in the after-disinfection plates of Scenario 2.

Another bacterium which was identified was *Staphylococcus hominis* which was isolated in one instance after disinfection plate of Scenario 2. Similar to *Staphylococcus epidermidis*, *Staphylococcus hominis* helps to eradicate *Staphylococcus aureus* on the skin by producing bactericidal molecules(21). *Staphylococcus hominis* hasa strong ability to form biofilms which make it less susceptible to disinfection(22).

Staphylococcus saprophyticus was identified on two TSA plates which failed disinfection. This organism normally resides in the gastrointestinal tract and urinary tract and is not as abundant on the skin as *Staphylococcus aureus* and *Staphylococcus epidermidis*, meaning it is less likely to contaminate blood products and be the causative agent of transfusion transmitted infections (23). However, some strains of *Staphylococcus saprophyticus* have the ability to form biofilms which make it resistant to disinfectants(24).

*Staphylococcus haemolyticus*is another skin commensal which was identified on one after disinfection plate of Scenario 1. Biofilm formation is a common phenotype of *Staphylococcus haemolyticus*which could be the reason why it survived disinfection(25). Farzad et al.(26) isolated *Staphylococcus haemolyticus*in a platelet unit. *Staphylococcus haemolyticus*is known for its genome

plasticity since which makes it highly resistant to multiple antibiotics; therefore, it would be difficult to treat a patient who is transfused with a blood product contaminated with this bacterium (27).

Although *Staphylococcus pasteuri*is not a common skin commensal, it has been isolated in one of the TSA plates that failed disinfection. It rarely causes diseases in humans; however, there was a case where it caused osteomyelitis and bacteraemia in a patient suffering from acute myeloid leukaemia (28). Therefore, it is possible that a patient transfused with a blood product contaminated with *Staphylococcus pasteuri*to develop bacteraemia.

Apart from staphylococci, other genera of bacteria that were identified are *Micrococcus* sp. and *Kocuria*sp. Micrococci are not as commonly found on the skin as staphylococci; however, Damgaard, et al. (29)isolated *Micrococcus luteus* in RCC and plasma units. *Kocuria palustris* can be found on the skin and mucous membranes and it is not usually pathogenic. However, bacteraemia caused by *Kocuriapalustris* in immunosuppressed individuals has been reported(30).

The bacterial colony on TSA plate labelled as 38C-3 had a 50% confidence interval between *Enhydrobacteraerosaccus*and *Moraxella osloensis.* This may be due to the high similarity in their 16s rRNA sequence and taxonomically considered to be the same species(31,32). One of the colonies on the TSA plate labelled 32D-2 could not be identified. Two TSA plates that did not reach the reduction target contained fungal growth. These were *Penicillium* sp. and *Cladosporium* sp. Both are skin commensals but *Penicillium* sp. is more abundant on the skin than *Cladosporium* sp. (33).

In their study, Opoku-Okrah et al.(34)disinfected the cord of the mother bag with 70% alcohol and obtained 3mls of blood which was cultured on 15mls of Brain-Heart Infusion broth contained in a sterile bottle. These were incubated at 37°C for 7 days. After overnight incubation, the broth was subcultured with a sterile loop on chocolate agar, MacConkey agar and blood agar every day for 3 consecutive days. The blood agar and MacConkey agar were incubated in aerobic conditions while the chocolate agar was incubated in anaerobic conditions. Similar to our findings, results showed that most bacteria isolated from the blood in the mother bag were coagulase negative staphylococci. Other bacteria which were less abundant were *Staphylococcus aureus*, *Corynebacterium diphteroids*, *Klebsiella pneumoniae*, and *Escherichia coli* (34). None of these bacteria were identified in the after disinfection plates of this study.

Patel et al. (3) swabbed the antecubital fossa of blood donors which were inoculated on blood agar media and incubated at 37°C for 24 hours. Bacterial colonies were subcultured on nutrient agar and on MacConkey agar. Bacteria which were isolated before skin disinfection were *Streptococcus* sp., *Micrococcus* sp., *Bacillus cereus*, *Bacillus megaterium*, *Staphylococcus epidermidis* and other staphylococci. Bacteria which survived the disinfection process were *Staphylococcus* sp., *Micrococcus* sp., and *Bacillus megaterium* (3). All bacteria which survived disinfection in said study were also identified in the after-disinfection plates of our study, except for *Bacillus* sp.

Celere et al. (7)swabbed the antecubital fossa of blood donors after disinfection with a sterile swab dipped in phosphate buffered saline and 3% Tween 80. Swabs were put in a sterile tube containing 1ml of 0.9% NaCl and inoculated on blood agar, mannitol salt agar and MacConkey agar. Bacteria which were identified were *Leuconostocmesenteroides, Staphylococcus cohnii*, *Staphylococcus hominis*, *Staphylococcus capitis*, and *Staphylococcus epidermidis* (7). *Leuconostocmesenteroides*and *Staphylococcus cohnii*were not identified in the after-disinfection plates of our study. Contrary to

UNDER PEER REVIEW

the findings of the current investigation, the most common bacterium identified in the after-disinfection plates was *Staphylococcus hominis*. Results showed that *Leuconostocmesenteroides*and *Staphylococcus hominis* were resistant to 10% P-I and 0.5% CHX disinfectants. No fungal growth was observed (7). Similar to the findings of the current investigation, a study conducted by Twoet al. (35)showed that the most common bacterium identified after cleaning the skin was *Staphylococcus epidermidis*. Bacteria were identified using 16S sequencing and their abundance was measured by quantitative polymerase chain reaction of DNA with universal primers for 16S ribosomal DNA (35).

Limitations

One of the limitations of the current study is that it was not possible to determine whether the presence of bacteria and fungi on the antecubital fossa resulted in contamination of blood products. Another limitation of this study is that a small sample size was used. A total of 50 blood donors were recruited in this study but only 47 blood donors were eligible for statistical analysis due to contamination of the TSA plates. As mentioned earlier, a small variation could negatively skew the results. For further investigations, it is suggested that warm water and hypoallergenic soap be used to clean the antecubital fossa prior to disinfection instead of hypoallergenic wipes. Additionally, to determine whether the bacteria present on the antecubital fossa after disinfection might have resulted in contamination of blood products, blood from the mother bag could be used to inoculate blood culture bottles and checked for sterility.

Conclusion

From the results obtained in this study, the difference between Scenario 1 and Scenario 2 is not significant; however, based on the current evidence cleaning reduces the bacterial load on the skin and helps the disinfection process be more effective. The act of cleaning also helps to eradicate those bacteria which are resistant to disinfection due to biofilm formation. These bacteria are also resistant to antibiotics which make it difficult to treat a patient who might be transfused with a contaminated blood product(11,16). The introduction of a cleaning step prior to disinfection is cost effective; however, it is time consuming. In conclusion, it is suggested that cleaning blood donors' skin would improve the effectiveness of the current disinfection protocol and can help minimise the risks of bacterial contamination within blood products.

UNDER PEER REVIEW

Consent

All participants where pseudo anonymous and couldn't be identified. Informed consent from the participants was granted during recruitment.

Ethical approval

Ethical approval for this research project was granted by the Faculty Research Ethics Committee (FHS-2023-00069).

References

1. Arghittu A, Dettori M, Deriu GM, Soddu S, Manca PC, Carboni AA, et al. Controlling Infectious Risk in Transfusion: Assessing the Effectiveness of Skin Disinfection in Blood Donors. Healthcare (Basel). 2022 May 5;10(5):845.

2. Tan KB, Chiu YC, Tham A, Hey M, Pang H, Leo QW, et al. Concentric Circles Painting Versus Back-and-Forth Scrubbing: A Comparison Between Two Skin Disinfection Techniques. IJRN. 2021 Nov 24;12(1):21–5.

3. Patel TG, Shukla RV, Gupte SC. Impact of Donor Arm Cleaning with Different Aseptic Solutions for Prevention of Contamination in Blood Bags. Indian Journal of Hematology & Blood Transfusion. 2012 Jan 31;29(1):17.

4. Fu L, Le T, Liu Z, Wang L, Guo H, Yang J, et al. Different efficacies of common disinfection methods against *candida auris* and other *candida* species. Journal of Infection and Public Health. 2020 May 1;13(5):730–6.

5. Rönner AC, Berland CR, Runeman B, Kaijser B. The hygienic effectiveness of 2 different skin cleansing procedures. J Wound Ostomy Continence Nurs. 2010;37(3):260–4.

6. Mihalache OA, Borda D, Neagu C, Teixeira P, Langsrud S, Nicolau AI. Efficacy of Removing Bacteria and Organic Dirt from Hands-A Study Based on Bioluminescence Measurements for Evaluation of Hand Hygiene When Cooking. Int J Environ Res Public Health. 2021 Aug 21;18(16):8828.

7. Celere MS, Ferreira O, Ubiali EMA, Julião FC, Fernandes AFT, de Andrade D, et al. Antimicrobial activity of two techniques for arm skin disinfection of blood donors in Brazil. Transfus Med. 2012 Apr;22(2):116–21.

8. Jensen DA, Macinga DR, Shumaker DJ, Bellino R, Arbogast JW, Schaffner DW. Quantifying the Effects of Water Temperature, Soap Volume, Lather Time, and Antimicrobial Soap as Variables in the Removal of Escherichia coli ATCC 11229 from Hands. J Food Prot. 2017 Jun;80(6):1022–31.

9. Debrincat A, Gialanze J, Spiteri N, Zammit V. A Procedure to Investigate the Efficacy of the Blood Donor Arm Disinfection Procedure for the Prevention of Blood Contamination. Journal of Pathology & Microbiology. 2021 Nov 18;3.

10. Basavarajegowda A, Harshavardhana, Anand DS. Effect of donor arm washing on microbial growth at phlebotomy site in first time blood donors. Hematology & Transfusion International Journal [Internet]. 2018 Nov 8 [cited 2024 Nov 7];Volume 6(Issue 6). Available from: https://medcraveonline.com/HTIJ/HTIJ-06-00188.pdf

11. Byrd AL, Belkaid Y, Segre JA. The human skin microbiome. Nat Rev Microbiol. 2018 Mar;16(3):143–55.

12. Brown MM, Horswill AR. Staphylococcus epidermidis—Skin friend or foe? PLoS Pathogens. 2020 Nov 12;16(11):e1009026.

UNDER PEER REVIEW

13. Joubert IA, Otto M, Strunk T, Currie AJ. Look Who's Talking: Host and Pathogen Drivers of Staphylococcus epidermidis Virulence in Neonatal Sepsis. International Journal of Molecular Sciences. 2022 Jan 13;23(2):860.

14. Greco C, Martincic I, Gusinjac A, Kalab M, Yang AF, Ramírez-Arcos S. Staphylococcus epidermidis forms biofilms under simulated platelet storage conditions. Transfusion. 2007 Jul;47(7):1143–53.

15. Martínez-Santos VI, Torres-Añorve DA, Echániz-Aviles G, Parra-Rojas I, Ramírez-Peralta A, Castro-Alarcón N. Characterization of Staphylococcus epidermidis clinical isolates from hospitalized patients with bloodstream infection obtained in two time periods. PeerJ. 2022;10:e14030.

16. Rozman U, Pušnik M, Kmetec S, Duh D, Šostar Turk S. Reduced Susceptibility and Increased Resistance of Bacteria against Disinfectants: A Systematic Review. Microorganisms. 2021 Dec 10;9(12):2550.

17. Chong CE, Bengtsson RJ, Horsburgh MJ. Comparative genomics of Staphylococcus capitis reveals species determinants. Front Microbiol. 2022;13:1005949.

18. Greco-Stewart VS, Ali H, Kumaran D, Kalab M, Rood IGH, de Korte D, et al. Biofilm formation by Staphylococcus capitis strains isolated from contaminated platelet concentrates. J Med Microbiol. 2013 Dec;62(12):1917.

19. Tran AQ, Topilow N, Rong A, Persad PJ, Lee MC, Lee JH, et al. Comparison of Skin Antiseptic Agents and the Role of 0.01% Hypochlorous Acid. Aesthet Surg J. 2021 Sep 14;41(10):1170–5.

20. Heath V, Cloutman-Green E, Watkin S, Karlikowska M, Ready D, Hatcher J, et al. Staphylococcus capitis: Review of Its Role in Infections and Outbreaks. Antibiotics (Basel). 2023 Mar 29;12(4):669.

21. Liu Y, Liu Y, Du Z, Zhang L, Chen J, Shen Z, et al. Skin microbiota analysis-inspired development of novel anti-infectives. Microbiome. 2020 Jun 5;8(1):85.

22. Mendoza-Olazarán S, Morfín-Otero R, Villarreal-Treviño L, Rodríguez-Noriega E, Llaca-Díaz J, Camacho-Ortiz A, et al. Antibiotic Susceptibility of Biofilm Cells and Molecular Characterisation of Staphylococcus hominis Isolates from Blood. PLoS One. 2015;10(12):e0144684.

23. Jones SA, Jones JM, Leung V, Nakashima AK, Oakeson KF, Smith AR, et al. Sepsis Attributed to Bacterial Contamination of Platelets Associated with a Potential Common Source - Multiple States, 2018. MMWR Morb Mortal Wkly Rep. 2019 Jun 14;68(23):519–23.

24. Hashemzadeh M, Dezfuli A a. Z, Nashibi R, Jahangirimehr F, Akbarian ZA. Study of biofilm formation, structure and antibiotic resistance in Staphylococcus saprophyticus strains causing urinary tract infection in women in Ahvaz, Iran. New Microbes New Infect. 2021 Jan;39:100831.

25. Fredheim EGA, Klingenberg C, Rohde H, Frankenberger S, Gaustad P, Flaegstad T, et al. Biofilm formation by Staphylococcus haemolyticus. J Clin Microbiol. 2009 Apr;47(4):1172–80.

26. Farzad BB, Farshad B, Zahra B, Nahid A, Mahsa KB. Bacterial contamination of platelet products in the Blood Transfusion Center of Isfahan, Iran. GMS Hyg Infect Control. 2016;11:Doc23.

UNDER PEER REVIEW

27. Eltwisy HO, Abdel-Fattah M, Elsisi AM, Omar MM, Abdelmoteleb AA, El-Mokhtar MA. Pathogenesis of Staphylococcus haemolyticus on primary human skin fibroblast cells. Virulence. 2020 Dec;11(1):1142–57.

28. Santoiemma PP, Kalainov DM, Mehta MP, Bolon MK. An unusual case of Staphylococcus pasteuri osteomyelitis. Infect Dis Rep. 2020 May 29;12(2):8523.

29. Damgaard C, Magnussen K, Enevold C, Nilsson M, Tolker-Nielsen T, Holmstrup P, et al. Viable bacteria associated with red blood cells and plasma in freshly drawn blood donations. PLoS One. 2015;10(3):e0120826.

30. Varghese G, Jamwal A, Sarawat D, Sahu C, Patel SS, Tejan N. Case Report: Case Series of Kocuria palustris Bacteremia among Immunocompromised Patients. Am J Trop Med Hyg. 2023 Nov 1;109(5):1113–7.

31. Kim JH, Son SM, Park H, Kim BK, Choi IS, Kim H, et al. Taxonomic profiling of skin microbiome and correlation with clinical skin parameters in healthy Koreans. Sci Rep. 2021 Aug 11;11(1):16269.

32. Li Z, Xia J, Jiang L, Tan Y, An Y, Zhu X, et al. Characterization of the human skin resistome and identification of two microbiota cutotypes. Microbiome. 2021 Feb 17;9(1):47.

33. Underhill DM, Iliev ID. The mycobiota: interactions between commensal fungi and the host immune system. Nat Rev Immunol. 2014 Jun;14(6):405–16.

34. Opoku-Okrah C, Feglo P, Amidu N, Dakorah MP. Bacterial contamination of donor blood at the Tamale Teaching Hospital, Ghana. Afr Health Sci. 2009 Mar;9(1):13–8.

35. Two AM, Nakatsuji T, Kotol PF, Arvanitidou E, Du-Thumm L, Hata TR, et al. The Cutaneous Microbiome and Aspects of Skin Antimicrobial Defense System Resist Acute Treatment with Topical Skin Cleansers. J Invest Dermatol. 2016 Oct;136(10):1950–4.

UNDER PEER REVIEW