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Evaluation of Antibacterial Efficacy of Commercially Available Gel-Based Hand Sanitizers and Alcohol-Based Hand Rubs Routinely Used in Dental Practice, in Relation to Different Handwashing Regimens

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Abstract

Background: The human skin acts as a reservoir of numerous microorganisms. Handwashing with soap and water removes excess organic matter and temporarily reduces the number of resident and transient flora.Alcohol-based hand rubs have been recommended for use in health care settings for hand hygiene. Other gel-based hand sanitizers have also recently made their way into the market. The study was conducted to compare the anti-bacterial effectiveness of commercially available gel-based hand sanitizers and alcohol-based hand rubs used in dental practice after various handwashing regimens. **Methodology:** An in vivo crossover study was conducted to assess the effectiveness of the commercially available gel-based hand sanitizers and alcohol-based hand rubs routinely used in dental practice. The study was conducted on twelve dental students.Bacterial samples were taken from each test person on the following occasions: before hand washing (baseline) and after each intervention – after washing hands with sterile distilled water, after washing hands with medicated soap, after using the test products and after using the test products without washing hands with water and soap. The samples were taken on blood agar plates by fingerprint contact sampling method. **Results:** There was a significant reduction seen in all the six intervention groups after washing hands with medicated soap and use of test products in comparison to baseline. After use of medicated soap, all the test products were equally effective. When the test products were used without washing hands with medicated soap and water, there was a difference in the antibacterial property of the test products. **Conclusions:** In a controlled hospital setting, when the hands are not visibly soiled or

contaminated, there is no significant difference in the percentage reduction of CFU counts between regimen 1 (washing hands with sterile distilled water, medicated soap and application of test products) and regimen 2 (direct application of test products without washing hands with soap and water)

Key words: Hand hygiene, hand sanitizers, antibacterial activity

Introduction

The human skin is a reservoir of numerous microorganisms that areeither pathogenic or commensal. In the year 1938, Price, classified the microorganisms recovered from hand into two broad categories: resident flora and transient flora. The resident flora permanently inhabitthe skin, are usually non-pathogenic, and colonize deeper layers of skin. These are more resistant to removal. The transient flora is mainly acquired from the environment or by direct contact with patients. They usually do not multiply on the skin, and colonize the superficial layers of skin. Transient flora are most often responsible for cross infections in hospital settings.(1)

Hand hygiene is a quintessential measure in reducing infections. Hand hygiene is a simple action, yet the lack of compliance among the healthcare providers in maintaining proper hand hygiene is a problem world-wide.(2) Gloving of hands is the most preferred universal barrier in dental practice to prevent microbial contamination from the hands of the operator. Despite numerous advances in techniques and technologies used for glove manufacturing, glove perforation rates have been reported to be as high as 17%.As the skin cannot be sterilized, it must be properly prepared.(3)

Hand washing with soap and water has traditionally been considered a measure of personal hygiene. The concept of cleansing hands with an antiseptic agent probably emerged in the early 19th century. Three main broad types of procedures can be employed for hand hygiene.(4,5)

1) Social Hand wash – using plain non-medicated soap.
2) Antiseptic and surgical hand wash – using medicated soap.
3) Hygienic and surgical hand disinfection – using antiseptic leave on preparation.

Handwashing with soap and water results in removal of excess organic matter and transient reduction in the number of resident and transient flora. Antiseptics enhance the antibacterial effect and results in elimination of transient flora.(6)

Alcohol-based hand rubs have routinely been recommended for hand hygiene in health care settings. Other gel-based hand sanitizers have also recently made their way into the market. With the advent of these gel-based hand sanitizers, there has been widespread acceptability and use of these products in daily life.

Furthermore, the onset of the COVID 19 pandemic has resulted in the prolific use of hand sanitizers amongst the public at large. There has been a tremendous increase in the manufacturing, sale, and use of hand sanitizers.

The study was conducted with the objective of comparing the anti-bacterial effectiveness of commercially available gel-based hand sanitizers and alcohol-based hand rubs used in dental practice with various hand washing regimens.

Methodology

An in vivo cross over study was planned to assess the effectiveness of the commercially available gel-based hand sanitizers and alcohol-based hand rubs routinely used in dental practice. The products used in the study and its composition are as follows.

1. Lifebuoy Total 10 hand sanitizer

Composition: Ethyl Alcohol 95% v/v IP, 62% w/w, Isopropyl Alcohol IP 10% w/w, 3%w/w niacinamide IP, 1% w/w, Perfumed gel base: q.s to 100% w/w.

2. Dettol aloe vera hand sanitizer

Composition: Alcohol IP (Denatured) eq to Absolute Alcohol- 72.34% w/w, Water PEG/PPG-17/6 copolymer, Propylene glycol, Acrylates/C10-30 alkyl acrylate, Tetrahydroxpropyl ethylenediamine, Aloe vera Gel, Perfume, Colour Tartrazine, and Brilliant Blue FCF

3. Savlon hand sanitizer

Composition: Ethanol IP 66.5% v/v, Isopropyl alcohol IP 3.5% v/v, Permitted colors, gel base.

4. Himalaya Pure Hands hand sanitizer

Composition: Dhanyaak (Coriandrum sativum), Nagaramusta (Cyperus scariosus), Ushira (Vetriveria zizanoides), Nimba (Azadirachta indica), Shati (Hedychium spicatum), processed in rectified spirit 60% w/w.

5. Sterillium

Composition: 1- Propanol and 2- Propanol with a total alcohol concentration of 75%, Mecetronium Ethyl Sulfate. 2% and Skin Conditioners.

6. NAP Hand Rub

Composition: Chlorhexidine gluconate 2.5% v/v, Isopropyl alcohol 70% v/v, Purified water, perfume.

Procurement of the test products

Lifebuoy total 10, Dettol Aloevera, Savlon, and Himalaya PureHands were the commercially available gel-based hand sanitizers. They were purchased from the

local stores. Sterillium and NAP hand rub were the alcohol-based hand rubs routinely used by the dental professionals in the region. These products were obtained from surgical supply stores.

Selection of study subjects, sample size and ethical clearance

The study was conducted on twelve dental students belonging to third year and final year undergraduate program, who gave voluntary informed consent to participate in the study. The sample size was obtained based on the results of a similar study. (6) Volunteers with skin infections and known hypersensitivity of any of the products used in the study were excluded. Necessary ethical clearance was obtained from the Institutional Review Board of the dental teaching institution, with approval number IEC/IGIDS/04/2021 dated 01.07.2021. prior to the start of the study. The procedures adhered to the ethical guidelines of the Declaration of Helsinki.

Study Design and regimen

The study was a cross-over study. The following were the interventions:

a. Washing hands with sterile distilled water without application of hand sanitizer/ alcohol-based hand rub.

b. Washing hands with sterile distilled water and medicated soap without application of hand sanitizer/ alcohol-based hand rub.

c. Washing hands with sterile distilled water with the application of hand sanitizer/alcohol-based hand rub.

d. Washing hands with sterile distilled water and medicated soap with the application of hand sanitizer/alcohol-based hand rub.

The following regimens were employed to assess the above - In the first regimen, the participant's fingerprint was taken at baseline, after washing with sterile distilled water, after handwashing with medicated soap, and after the use of each test product. In the second regimen, the participant's fingerprint was taken at baseline and after the use of each test product.

In all the study regimens, the twelve study subjects washed their hands with sterile distilled water as per the WHO recommendation for hand hygiene. After hand washing, the excess water was shaken off and dried. The sample collections were performed between 8 am and 9 am every day and the study volunteers were asked to refrain from use of any hand sanitizers prior to the sampling, to avoid any residual or carry over effect of other sanitizers. To ensure uniformity in handwashing procedure and the use of medicated soap and test products, the study volunteers were trained as per recommendations form World Health Organization. A triple blind study design was followed.

The hand sanitizers and alcohol-based hand rubs were used as per the manufacturer's instructions and the hands were rubbed together until they were dry and left free of contamination for 2 minutes before bacterial sampling. The commercially available concentrations of the antiseptic solutions were used without any dilution.

Bacterial Sampling

Bacterial samples were taken from each test person on the following occasions: before hand washing (baseline) and after each intervention – after washing hands with sterile distilled water, after washing hands with medicated soap, after using the test products and after using the test products without washing hands with water and soap. The samples were taken on blood agar plates by fingerprint contact sampling method. The plates were incubated aerobically at 37°C for 24 hours.(6)

Quantitative determination of the antibacterial effect: The number of colony-forming units (CFU's) was counted from samples obtained. To ensure uniformity in area of focus, squares of 1 sq. cm. area were marked on the culture plates against the fingerprints and the number of colonies within each square was counted. The colony-forming units were counted using the colony-forming units counter. All counting was performed by a single person. (6)

Statistical Analysis: Mean and standard deviation of different samples were tabulated. Statistical significance was measured by using one-way ANOVA followed by Tukey's post hoc test. p-value <0.05 was considered statistically significant. The analysis of data was done by Statistical Package for Social Sciences (SPSS) Version 19.0 for Windows.

Results

The study was conducted among 12 volunteers who gave voluntary informed consent for participation. The study was conducted across a period of 2 months. The results were as follows.

It was observed that there was no significant difference in the CFU counts between the various intervention groups of both regimens. ($p = 0.200$ and $p = 0.553$ respectively).The comparison of colony forming unit counts after washing hands with sterile distilled water in comparison to baseline in all the six intervention groups revealed no significant reduction in bacterial count

Table 1 shows the comparison of colony forming unit counts in each of the intervention groups at baseline and after washing hands with medicated soap. It is observed that there was a significant reduction seen in all the six intervention groups after washing hands with medicated soap in comparison to baseline.

Table 2showsthe comparison of colony forming unit counts after the use of the commercially available gel-based hand sanitizers and alcohol-based hand rubs, following washing of hands with medicated soap, with baseline. It is observed that there was a significant reduction in the colony forming unit counts in all the six intervention groups compared to baseline.

Table 3shows the comparison of colony forming unit counts after use of the commercially available gel-based hand sanitizers and alcohol-based hand rubs, without washing hands with medicated soap and water. It is observed that there has been a highly significant reduction in the colony counts in each of the six intervention groups compared to baseline.

Table 4shows the comparison on colony forming unit counts after hand washing with sterile distilled water and handwashing with medicated soap. It was observed that there was a significant reduction in the colony counts after the use of medicated soap.

Table 5showsthe comparison of colony forming unit counts after hand wash with medicated soap and use of commercially available gel-based hand sanitizers and alcohol-based hand rubs. A significant reduction was observed in each of the intervention groups after the use of test products.

Table 6shows the comparison of colony forming unit counts between the intervention groups after each handwashing regime. It is observed that there was no statistically significant difference in the colony counts between the 6 groups at baseline, after hand wash with sterile distilled water, after handwash with medicated soap and after handwash with medicated soap followed using test products.

Table 7compares the colony forming unit counts between various intervention groups at baseline and after use of test products without handwashing with medicated soap and water. It is observed that there was no significant difference in the colony forming unit counts at baseline, but a statistically significant difference ($p=0.022$) was observed between the groups after the use of test products without handwashing with medicated soap and water. Post hoc analysis revealed that the maximum reduction in CFU counts was observed with the use of Sterillium, comparable to NAP hand rub, Savlon gel and Himalaya pure hands. The reduction demonstrated by Lifebuoy total 10 and Dettol aloe vera were significantly lower than the other 4 products.

Table 8compares the percentage reduction in the colony forming unit counts at the end of each regimen (use of test products after washing hands with soap and water; and use of test products directly, without use of soap and water). It is observed that there was no significant difference in the percentage reduction

of colony forming unit counts, between the endpoints of both regimens,in any of the groups.

Discussion

Maintaining a good hand hygiene is important to prevent healthcare-associated infections. Hand hygiene remains one of the most effective methods of preventing such infections. However, factors such as the cleansing agent, duration of hand hygiene and selection of appropriate agent decides its effectiveness.

Hand hygiene is a basic requirement for every healthcare personnel in a hospital setting today. The increasing numbers of nosocomial infections and their complications is preventable by increasing the awareness about hand hygiene practices. (7)

Infection control in dentistry is an ever-growing perturbation. Patients undergoing dental treatment are at high-risk. An equal concern has been exhibited for cross-contamination and disease transmission from patient to patient. The Centre for Disease Control (CDC), in its infection control guidelines, indicated that even dental impressions are potential sources of cross-contamination and should be handled in a manner that prevents exposure to practitioners, patients, and the environment. Initially, the dentistry was routinely done without protective gears but after 1991 dental personnel were required to wear gloves, masks,gowns, and protective eyewear.(8)Meticulous hand hygiene is quintessential in dentistry.

While sanitizing hand lotions has become increasingly popular, studies show that good handwashing with soap and water is still more effective if you have visibly soiled hands. Hand sanitizer is not effective for hands that are visibly soiled.(9)Practicing hand hygiene is a simple yet effective way to prevent infections. Cleaning your hands can prevent the spread of germs, including those that are resistant to antibiotics and are becoming difficult, if not impossible, to treat.(10)

Since alcohols have excellent activity and the most rapid bactericidal action of all antiseptics, they are the preferred agents for hygienic hand rubs, so called "waterless hand disinfection.", especially when hands are not visibly soiled. In addition, alcohols are more convenient than aqueous solutions for hygienic hand rubs because of their excellent spreading quality and rapid evaporation. Alcohol-based hand rubs are well suited for hygienic hand disinfection for the following reasons: optimal antimicrobial spectrum (active against all bacteria and most clinically important viruses, yeasts, and fungi); no wash basin necessary for use and easy availability at the bedside; no microbial contamination of health-care workers' clothing; and rapidity of action.(6)

The culture of organisms such as Streptococcus pneumoniae, Streptococcus pyogenes, and Staphylococcus aureus is considered as the 'benchmark' for the diagnosis of serious bacterial infections. The isolation of some organisms requires a source of blood as a culture medium supplement. Blood agar, containing general nutrients, is useful for cultivating fastidious organisms and for determining the haemolytic capabilities of an organism(11). Hence blood agar was used in the study.

The use of hand sanitizers and solutions and gels became widespread amongst the public after the onset of COVID-19 pandemic in the beginning of the year 2020. The use and availability of hand sanitizers were significantly lesser among the general population prior to the pandemic. The use of the sanitizers was largely restricted to health care professionals. Although several brands of sanitizers in liquid/gel/foam form are currently available with varied ingredients, the availability of brands during the submission of proposal for this study (ICMR STS 2020), was very limited. Hence, the present study included those brands (Himalaya Pure Hands, Lifebuoy Total 10, Savlon and Dettol with aloe vera) that were commercially available over the counter, prior to the onset of COVID 19 pandemic. Sterillium and NAP Hand rub was commonly used in health care settings in the locality and hence were included in the study.

The study was conducted with regimens that explored the various combination methods of hand hygiene, practiced by the health care workers. Hence comparison between handwashing with or without medicated soap and with or without use of hand sanitizers were performed. Hence the study was divided into 2 regimens. The first assessed the colony forming counts at baseline, followed by assessment of counts after washing with sterile distilled water, followed by hand washing with medicated soap and use of the test products. The second assessed the baseline counts followed by counts after the use of test products.

Handwashing practice is difficult to assess but the microbiological analysis of hands shows promise as an indicator of this behaviour. Finger-print contact sampling method of assessing the total bacterial count is a semi-quantitative method of measuring the contamination. It is a simpler and cost-effective method to assess contamination by pressing into selective agar plates.(12,13)

It is observed that there was no significant difference in the CFU counts between the various intervention groups of both regimens. Hence this ensures comparability within and between the groups after each regimen.

The comparison of colony forming unit counts after washing hands with sterile distilled water in comparison to baseline in all the six intervention groups, revealed that, although there was a reduction in the counts after handwash in all the six groups compared to baseline, the reduction was not statistically significant. A study conducted in London in 2011 by Burton M et. al. showed a

significant reduction in total bacterial count after handwashing alone. However, the study was conducted after deliberately contaminating the hands of volunteers.(13)

The comparison of colony forming unit counts in each of the intervention groups at baseline and after washing hands with medicated soap showed that there was a significant reduction seen in all the six intervention groups after washing hands with medicated soap in comparison to baseline. The results are consistent studies conducted by Burton M et. al. in London in 2011(13), De Alwis et. al. in Malaysia in 2012(14) and Alsager et. al. in Tripoli in 2018(15). The results are in contrast with the studies conducted by Subramaniam R et. al. in Davangere in 2015(6) and Myklebust S in Norway in 1985(1). The possible reason for this contrasting result could be due to the use of sterile distilled water in our study compared to tap water in the other two studies. Also, the authors of these studies attribute the increase in counts due to mobilization of bacteria from deeper layers of skin during the process of handwashing with antiseptic soap.

The comparison of colony forming unit counts after the use of the commercially available gel-based hand sanitizers and alcohol-based hand rubs, following washing of hands with medicated soap, with baseline, demonstrated a significant reduction in the colony forming unit counts in all the six intervention groups compared to baseline. The sanitizers used in the study (except Himalaya pure hands), had one or more of the following alcohols - ethyl alcohol, isopropyl alcohol, absolute alcohol, propylene glycol, 1 propanol, 2 propanol or non-alcoholic chlorhexidine gluconate; as active ingredients. There is substantial evidence supporting the antibacterial activity of these ingredients.(1,3,6,16–18,18–20) Ethanol, isopropyl alcohol and n propanol act by denaturing of protein in plasma membrane of bacteria, inhibition or uncoupling of mRNA and protein synthesis through effects on ribosomes and RNA polymerase, or associated with protein denaturation.(21)

Himalaya pure hands is herbal sanitizer that contains *Coriandrum sativum*, *Cyperus scariosus*, *Vetriveria zizanoides* and *Azadiracta indica* along with rectified spirit. The antimicrobial activity of PureHands Herbal Hand Sanitizer is indicative of the additive role of *Coriandrum sativum, Vetiveria zizanioides*, and *Azadirachta indica* in addition to alcohol. Neem *(Azadirachta indica)* is recognized as a medicinal plant well known for its antibacterial, antimalarial, antiviral, and antifungal properties.(22)Neem contains different active phytoconstituents such as alkaloids, glycosides, trepenoids, steroids and tannins.(23) Studies have demonstrated broad antibacterial activity of Coriandrum sativum and Veriveria zizanoides essential oils.(24,25)

When the CFU counts at baseline and after the use of test products (without handwashing with water and soap), there was no significant difference in the colony forming unit counts at baseline, but a statistically significant difference

(p=0.022) was observed between the groups after the use of test products. Post hoc analysis comparing the effectiveness of various test products revealed that the maximum reduction in CFU counts was observed with the use of Sterillium, comparable to NAP hand rub, Savlon gel and Himalaya pure hands. The reduction demonstrated by Lifebuoy total 10 and Dettol aloe vera were significantly lower than the other 4 products. Various other studies have shown a high antibacterial efficacy demonstrated by Sterillium compared to Lifebuoy and Dettol hand sanitizers in comparison with other hand sanitizers.(3,6,17)

When the hands are not visibly soiled or contaminated, there is no significant difference in the percentage reduction of CFU counts between regimen 1 (washing hands with sterile distilled water, medicated soap and application of test products) and regimen 2 (direct application of test products without washing hands with soap and water). This observation is in contrast with the study conducted by Khairnar et al in Sangli(16), where the combination of hand washing with soap and water followed by the use of sanitizers resulted in significant reduction in CFU counts compared to direct use of hand sanitizers. One possible reason for this difference could be due to the fact that in the study conducted in Sangli, the dental students were deliberately asked to contaminate the hands, that could have resulted in soiling of hands.

Impact of the study and recommendations

The study focuses on comparison of antibacterial efficacy of the test products with or without handwash with soap and water. Although there is considerable evidence regarding the lack of effectiveness of using hand sanitizer alone when the hands are visible soiled or contaminated, in health care settings, there is a better hygienic atmosphere and there is a prolific use of hand sanitizers or hand rubs without washing with soap and water. Given the study setting, the study reveals that Sterillium, NAP hand rub, Savlon gel and Himalaya pure hands demonstrated a better antibacterial efficacy than Lifebuoy total 10 and Dettol aloe vera. Cost is an important factor that aids the selection of these products.

One of the limitations of this study was that this study assessed the total colony forming unit count on blood agar. This may include both pathogenic and non-pathogenic bacteria and both resident and transient flora of hands. Further research can be carried out for assessing the antibacterial activity on selected pathogenic flora, that are responsible for the nosocomial infections. Yet another limitation is that the study was conducted among dental students – a-controlled hospital setting. Although this reflects the settings in healthcare sector, the impact of the test products in visibly soiled or contaminated hands in community setting can further aid in selection of the over-the-counter hand sanitizers for the general public. A further extension of this research in that direction is also recommended.

Conclusions

The study assessed the antibacterial efficacy of commercially available gel-based hand sanitizers and alcohol-based hand rubs routinely used in dental practice, in relation to different handwashing regimens. The following were the conclusions drawn:

a. It was observed that although there was a reduction in the counts after handwash with sterile distilled water in all the six groups compared to baseline, the reduction was not statistically significant.

b. There was a significant reduction seen in all the six intervention groups after washing hands with medicated soap in comparison to baseline.

c. The comparison of CFU counts after the use of test products after washing of hands with medicated soap, with baseline demonstrated a significant reduction in the colony forming unit counts in all the six intervention groups compared to baseline.

d. When the test products were used without washing hands with medicated soap and water, there was a highly significant reduction in the colony counts in each of the six intervention groups compared to baseline.

e. Comparison of CFU counts after hand washing with sterile distilled water and handwashing with medicated soap revealed that there was a significant reduction in the colony counts after the use of medicated soap.

f. There was a significant reduction in CFU counts after hand wash with medicated soap and the use of test products in all the groups.

g. Intergroup comparison revealed no statistically significant difference in the colony counts between the 6 groups at baseline, after hand wash with sterile distilled water, after handwash with medicated soap and after handwash with medicated soap followed using test products. This suggest that the antibacterial efficacy remained the same for all the test products when used after washing the hands with soap and water.

h. When the test products were used directly without handwash with soap and water, it was observed that there was no significant difference in the colony forming unit counts at baseline, but a statistically significant differencewas observed between the groups after the use of test products. Post hoc analysis revealed that the maximum reduction in CFU counts was observed with the use of Sterillium, comparable to NAP hand rub, Savlon gel and Himalaya pure hands. The reduction demonstrated by Lifebuoy total 10 and Dettol aloe vera were significantly lower than the other 4 products.

i. In a controlled hospital setting, when the hands are not visibly soiled or contaminated, there is no significant difference in the percentage reduction of CFU counts between regimen 1 (washing hands with sterile distilled water, medicated soap and application of test products) and regimen 2 (direct application of test products without washing hands with soap and water)

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Table 1: CFU counts after hand washing with medicated soap compared to baseline in the six intervention groups.

Group	**CFU count (at baseline)**	**CFU count (after handwash with medicated soap)**	**Paired t test**
Sterillium	23.08 ± 20.70	3.17 ± 2.40	p = 0.010*
NAP Hand rub	19.75 ± 11.49	6.75 ± 8.66	p = 0.003*
Savlon gel	27.50 ± 23.88	9.00 ± 7.49	p = 0.017*
Himalaya pure hands	22.00 ± 20.79	8.58 ± 7.77	p = 0.037*
Lifebuoy total 10	43.17 ± 42.01	14.92 ± 17.26	p = 0.029*
Dettol Aloe vera gel	34.33 ± 21.78	8.33 ± 7.27	p = 0.002*

* (Significant at p = 0.05)

Table 2: CFU counts after using test products following hand washing with medicated soap compared to baseline in the six intervention groups.

Group	CFU count (at baseline)	CFU count (after handwash with medicated soap followed by use of test products)	Paired t test
Sterillium	23.08 ± 20.70	1.67 ± 1.96	p = 0.006*
NAP Hand rub	19.75 ± 11.49	2.08 ± 2.42	p < 0.001*
Savlon gel	27.50 ± 23.88	2.42 ± 5.05	p = 0.003*
Himalaya pure hands	22.00 ± 20.79	1.50 ± 3.14	p = 0.007*
Lifebuoy total 10	43.17 ± 42.01	3.92 ± 6.20	p = 0.009*
Dettol Aloe vera gel	34.33 ± 21.78	3.50 ± 3.11	p < 0.001*

* (Significant at p = 0.05)

Table 3: CFU counts after using the test products without handwashing compared to baseline in the six intervention groups.

Group	CFU count (at baseline)	CFU count (after use of test products)	Paired t test
Sterillium	34.58 ± 34.97	0.92 ± 1.56	p = 0.006*
NAP Hand rub	18.83 ± 24.01	1.08 ± 2.39	p = 0.020*
Savlon gel	28.17 ± 18.86	1.17 ± 2.85	p < 0.001*

Himalaya pure hands	27.33 ± 31.55	2.75 ± 3.25	p = 0.015*
Lifebuoy total 10	22.08 ± 16.23	3.75 ± 6.26	p = 0.003*
Dettol Aloe vera gel	34.75 ± 17.81	8.17 ± 11.43	p < 0.001*

* (Significant at p = 0.05)

Table 4: CFU counts after hand washing with sterile distilled water and hand washing with medicated soap, in the six intervention groups

Group	**CFU count (at baseline)**	**CFU count (after handwash with medicated soap)**	**Paired t test**
Sterillium	19.92 ± 20.61	3.17 ± 2.40	p = 0.016*
NAP Hand rub	16.92 ± 16.74	6.75 ± 8.66	p = 0.021*
Savlon gel	15.42 ± 13.75	9.00 ± 7.49	p = 0.045*
Himalaya pure hands	15.75 ± 15.33	8.58 ± 7.77	p = 0.047*
Lifebuoy total 10	18.08 ± 16.83	14.92 ± 17.26	p = 0.049*
Dettol Aloe vera gel	20.83 ± 13.85	8.33 ± 7.27	p = 0.013*

* (Significant at p = 0.05)

Table 5: CFU counts after hand washing with medicated soap and the use of test products in the six intervention groups

Group	**CFU count (after handwash with medicated soap)**	**CFU count (after handwash with medicated soap followed by use of test**	**Paired t test**

		products)	
Sterillium	3.17 ± 2.40	1.67 ± 1.96	p = 0.045*
NAP Hand rub	6.75 ± 8.66	2.08 ± 2.42	p = 0.044*
Savlon gel	9.00 ± 7.49	2.42 ± 5.05	p = 0.003*
Himalaya pure hands	8.58 ± 7.77	1.50 ± 3.14	p = 0.012*
Lifebuoy total 10	14.92 ± 17.26	3.92 ± 6.20	p = 0.026*
Dettol Aloe vera gel	8.33 ± 7.27	3.50 ± 3.11	p = 0.025*

* (Significant at p = 0.05)

Table 6: Comparison of CFU counts between the intervention groups after each handwashing regime

Group	**Baseline**	**After hand wash with sterile distilled water**	**After handwash with medicated soap**	**After hand wash with medicated soap followed by use of test products**
Sterillium	23.08 ± 20.70	19.92 ± 20.61	3.17 ± 2.40	1.67 ± 1.96
NAP Hand rub	19.75 ± 11.49	16.92 ± 16.74	6.75 ± 8.66	2.08 ± 2.42
Savlon gel	27.50 ± 23.88	15.42 ± 13.75	9.00 ± 7.49	2.42 ± 5.05
Himalaya pure hands	22.00 ± 20.79	15.75 ± 15.33	8.58 ± 7.77	1.50 ± 3.14
Lifebuoy total 10	43.17 ± 42.01	18.08 ± 16.83	14.92 ± 17.26	3.92 ± 6.20

Dettol Aloe vera gel	34.33 ± 21.78	20.83 ± 13.85	8.33 ± 7.27	3.50 ± 3.11
ANOVA	p = 0.200	p = 0.952	p = 0.104	p = 0.587
Post Hoc	Not Significant	Not Significant	Not Significant	Not Significant

* (Significant at p = 0.05)

Table 7: Comparison of CFU counts between the intervention groups at baseline and after use of test products without handwashing with medicated soap and water

Group	**Baseline**	**After use of test products without hand washing with medicated soap and water**
Sterillium	34.58 ± 34.97	0.92 ± 1.56
NAP Hand rub	18.83 ± 24.01	1.08 ± 2.39
Savlon gel	28.17 ± 18.86	1.17 ± 2.85
Himalaya pure hands	27.33 ± 31.55	2.75 ± 3.25
Lifebuoy total 10	22.08 ± 16.23	3.75 ± 6.26
Dettol Aloe vera gel	34.75 ± 17.81	8.17 ± 11.43
ANOVA	p = 0.553	p = 0.022
Post Hoc	Not Significant	Sterillium = NAP Hand rub = Savlon gel = Himalaya Pure hands < Lifebuoy total 10 < Dettol Aloe vera

* (Significant at p = 0.05)

Table 8: Comparison of percentage reduction of CFU counts after each regimen in each group.

Group	Regimen 1 percentage reduction	Regimen 2 percentage reduction	t test
Sterillium	86.50 ± 20.74	96.58 ± 8.25	p = 0.132
NAP Hand rub	88.58 ± 15.64	93.83 ± 14.56	p = 0.404
Savlon gel	91.58 ± 21.10	95.83 ± 8.10	p = 0.521
Himalaya pure hands	89.00 ± 24.86	89.25 ± 12.72	p = 0.976
Lifebuoy total 10	80.42 ± 29.40	89.75 ± 15.96	p = 0.344
Dettol Aloe vera gel	84.75 ± 18.34	80.00 ± 25.58	p = 0.606

* (Significant at p = 0.05)

Regimen 1 - Handwash with distilled water + medicated soap + use of test product

Regimen 2 - Direct application of test product without handwash with soap and water