Assessment of skin microbiota and biometric parameters: a comprehensive comparison of four types of hand cleansers

Setareh Ameri, MSc [1]
Saman Ahmad Nasrollahi, PhD [1*]
Aniseh Samadi, PhD Candidate [1]
Fatemeh Amiri, MSc [1]
Solmaz Ahmadvand, PharmD [2]
Somayeh Yadangi, MSc [1]
Mahsa Fattahi, MD [1]
Marzieh Ehsani, MD [1]
Alireza Firooz, MD [1]

1. *Pharmaceutical, Cosmeceutical and Hygienic Products Microbial Control Lab, Center for Research & Training in Skin Diseases & Leprosy, Tehran University of Medical Sciences, Tehran, Iran*
2. *Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran*

**Corresponding author:*
Saman Ahmad Nasrollahi, PharmD, PhD
Center for Research & Training in Skin Diseases & Leprosy (CRTSDL), Tehran University of Medical Sciences (TUMS), 415 Taleqani Ave, Tehran, Iran
Email: snasrollahi@tums.ac.ir

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Background: Hand hygiene plays a vital role in controlling pathogenic microorganisms' transmission and maintaining the interaction between skin microbiota and biometric parameters. This study evaluated the effects of hand cleansers including alcoholic gel, alcoholic pad, antibacterial, and simple wipes on skin biometric parameters and microbiota.

Methods: Samples were collected from the hands of 15 healthy office workers with a mean age of 37.70 ± 9.6 years. Then, the subjects were instructed to use cleansers in four following rounds, with a one-week washout period between the rounds. Sample collection was performed before, right after, and one hour after using the cleansers. Microbial isolates were investigated via standard microbiological techniques, and biometric measurements were made using the Cutometer® MPA 580. The obtained data were analyzed using the paired t-test and repeated measures ANOVA.

Results: Overall, there were no significant differences between cleansers in reducing the total aerobic microbial count (TAMC). Also, there was an approximate return to the initial count of resident microbiota one hour after using the alcoholic pad. A significant difference was observed in decreasing the *Staphylococcus aureus* count using antibacterial wipes rather than simple wipes. Simple wipes had the most increasing effect on transepidermal water loss (TEWL), showing a significant difference with the alcoholic gel. Furthermore, alcoholic gel caused a greater pH decrease in comparison to other products.

Conclusion: Alcoholic cleaners are more effective than antibacterial and simple wipes due to maintaining the skin's biometric parameters. An additional advantage is that alcoholic pads can preserve the resident microbiota.

Keywords: hygiene, alcoholic cleaner, microbiota

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INTRODUCTION

Hand hygiene plays a key role in preventing the transmission of pathogenic organisms in the community [1]. The importance of hand hygiene is due to the dispersion of microorganisms in the environment, where the hand can act as a mediator to transmit colonized microorganisms to and from others [2].

Office workers are constantly in contact with equipment such as desks, computers, and phones, which are potentially at risk of transmission

of contaminations from the environment. This condition requires searching for more effective and convenient hand hygiene products instead of basic water and soap [3,4]. Antimicrobial products (gel, solution, and wipes) and simple wipes are widely used for post-contamination hand hygiene. Alcoholic cleansers are considered one of the safest agents available without having any toxic effects on the skin. Non-alcoholic hand sanitizers, which sometimes contain quaternary ammonium-type compounds, are extensively used as an alternative to soap and water for hand cleaning [3,5].

The skin of the hand is colonized by two groups of microflora known as resident and transient microbiota. Under normal conditions, none are pathogenic, and the microbiota contributes to skin homeostasis and modulates immune responses [6]. Dysbiosis and disrupted homeostasis result from an imbalance between resident microbiota and the immune response, affecting the pathogenicity of transient microbiota [6,7]. Moreover, the integrity of the epidermal barrier depends on the maintenance of appropriate biometric parameters against environmental factors. Therefore, changes in skin microbiota and biometric parameters are considered a turning point to induce various skin disorders and diseases [7,8].

There is no need to use only strong antiseptic agents for hand hygiene; a product with the least effect on resident microbiota and biometric parameters of the skin should be chosen to maintain homeostasis [7,8]. In addition, several factors such as being fast-acting, time-saving, and broad-spectrum are favored [9]. In light of the mentioned issues, the present study evaluated and compared the effects of four types of antiseptics and simple cleansers (alcoholic gel, alcoholic pad, antibacterial wipe, and simple wipe) on the skin biometric parameters and microbiota.

PARTICIPANTS AND METHODS

Study design and ethics

The present study was conducted in the Center for Research and Training in Skin Diseases and Leprosy, Tehran University of Medical Sciences, from March to September 2017.

The participants voluntarily entered and were allowed to leave the study at any time. Furthermore, the information about all cleansers was shared with them. The volunteers' personal information was reserved in accordance with the ethical principles provided by the Good Clinical Practice (GCP), and all of them provided written informed consent. All forms and study protocols were accepted by the institutional ethics committee (IR.TUMS.VCR.REC.1396.3220).

The inclusion criteria were healthy volunteers from an administrative division, use of the recommended cleanser during the intervention, and signing the informed consent form. The exclusion criteria were any wounds or injuries in the dominant hand, allergy to the studied cleansers, use of topical and oral antibiotics, use of hand moisturizers, diseases such as diarrhea or the common cold during the intervention, having long or artificial nails, and applying nail polish. After evaluating the inclusion and exclusion criteria, 15 healthy office workers aged 18 to 59 years were enrolled in the study to use each product in four following rounds, with a one-week washout period between the rounds.

All the necessary assessments were conducted before, right after, and one hour after interventions, referred to in order as the first, second, and third visits.

Hand hygiene products

The four tested cleansers were alcoholic gel containing ethanol 70% (V/V) (Delban Co., Iran), alcoholic pad containing 70% ethanol (Newsaad Co., Iran), antibacterial hand sanitizer wet wipe containing 0.13% benzalkonium chloride (Uni Led Co., Iran), and simple wipe free from any antiseptic agents (Pakan Co., Iran).

Intervention

We aimed to evaluate the effect of the studied cleansers on the reduction of the skin microbiota and the change in biometric parameters such as transepidermal water loss (TEWL), skin pH, and hydration. The participants were asked to wash their hands with non-medicated soap before using the cleansers. They were required to work in their office regularly using the keyboard, telephone, and other administrative equipment for two hours. Then, they cleaned their hand with the identified

cleanser as instructed by lab technicians. Afterward, they continued their routine work for one hour.

Microbial assessments

The volunteers' dominant hand was sampled by a sterile swab soaked in tryptic soy broth (TSB; Liofilchem, Italy) before the intervention, immediately after using the cleansers, and one hour later according to the protocol.

The samples were diluted up to 0.01 solutions and inoculated onto plates containing tryptic soy agar (TSA; Liofilchem, Italy), MacConkey agar (Liofilchem, Italy), mannitol salt agar (MSA; Liofilchem, Italy), bile aesculin azide agar (Biolife, Italy) and Sabouraud dextrose agar (SDA; Liofilchem, Italy). All plates were incubated at 37 °C for 24 to 48 hours, except the SDA medium, which was incubated at room temperature for 48 to 72 hours. Standard microbiological techniques were used to identify the isolated microorganisms, and their counts were statistically compared [10].

Assessment of the skin's biometric parameters

Three important parameters, including TEWL, skin pH (dorsal and palmar of hands), and hydration, were investigated. These biometric parameters were measured using the Cutometer® MPA 580 (Courage & Khazaka electronic GmbH, Cologne, Germany) [11] before, right after, and one hour after intervention under standard and constant conditions of temperature (20 ± 1 °C) and humidity (30-40%). Before evaluation, all the volunteers stayed in the lab for approximately 20 minutes for adaptation.

Statistical analysis

The obtained data were analyzed using the IBM SPSS Statistics software version 20 (IBM Corp., Armonk, NY, USA). The paired t-test was used to analyze the variables with normal distribution, and a P-value < 0.025 was considered significant. These results were reported as mean ± standard deviation (SD). The comparison between four cleansers was performed using repeated measurements ANOVA test, and a P-value < 0.05 was considered significant.

RESULTS

Fifteen healthy office workers (13 females and 2 males) with a mean age of 37.70 ± 9.6 years were enrolled in the study. As depicted in Figure 1a, the mean TAMC in both alcoholic gel and alcoholic pad groups decreased significantly right after usage compared to before usage (P = 0.002 and 0.001, respectively). There was a non-significant reduction at the third visit in the alcoholic gel. In addition, one hour later, in the alcoholic pad, the bacteria grew again and reached the level before intervention. In the groups of antibacterial and simple wipes, there were non-significant decreases in the TAMC right after usage. This reduction continued in the simple wipe at the third visit, while the TAMC increased in the antibacterial wipe group.

In terms of the effects of antiseptics and simple cleansers on coagulase-negative staphylococci (CoNS) (Figure 1b), alcoholic gel showed a significant reduction at the second visit (P = 0.017) as well as a non-significant decrease at the third visit. The CoNS count decreased significantly right after using an alcoholic pad (P = 0.022). This count increased one hour later and returned to the count of the first visit. In antibacterial and simple wipes, the CoNS count decreased at the second visit compared to before usage, though the changes were not significant. At the third visit, CoNS increased to more than before usage in the antibacterial wipe, though this was not significant. In the simple wipe, a non-significant decrease of CoNS was observed.

The count of Gram-negative bacteria (GNB) decreased at the second visit across all products. These bacteria increased one hour after using alcoholic gel and alcoholic pad, but the decremental trend continued in antibacterial and simple wipes. In all visits, there were no significant changes in GNB (Figure 1c).

As Figure 1d depicts, right after using alcoholic gel, alcoholic pad, and antibacterial wipe, a significant decrease in the count of *Staphylococcus aureus* (P = 0.003, 0.001, and 0.001, respectively) was observed. This significant reduction continued in the antibacterial wipe group one hour later (P = 0.018). The results in the group of simple wipes were inconsistent with those of the antiseptic products, and a non-significant increase in the count of *S.*

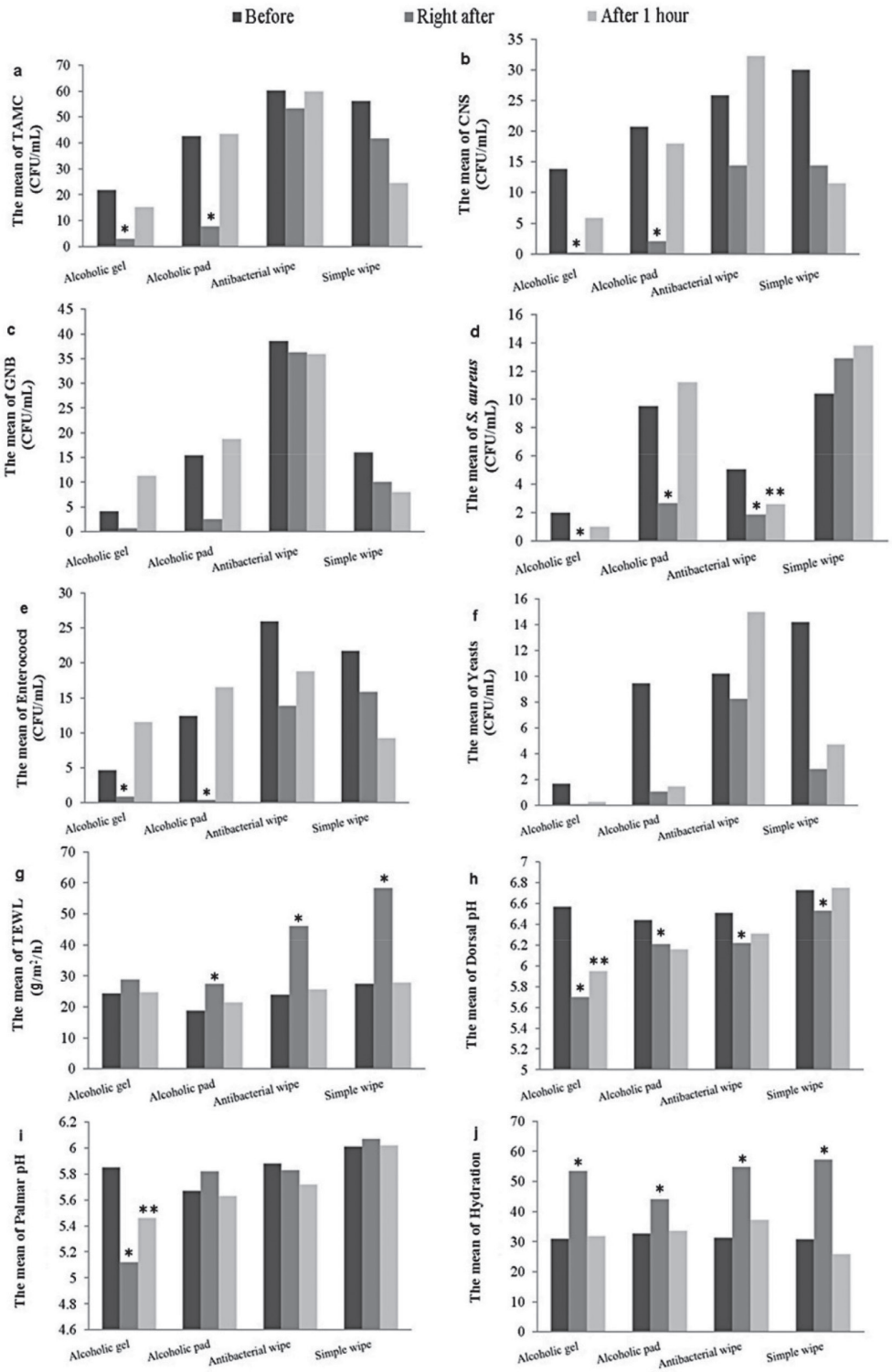

Figure 1. The alteration in the viability of skin microbiota and biometric parameters including TAMC (a), CNS (b), GNB (c), *S. aureus* (d), enterococci (e), yeasts (f), TEWL (g), dorsal pH (h), palmar pH (i) and hydration (j) before, right after, and one hour after treatment with alcoholic gel, alcoholic pad, antibacterial wipe, and simple wipe.
**P*-values of differences between first and second visits < 0.025. ***P*-values of differences between first and third visits < 0.025.
TAMC, total aerobic microbial count; CNS, coagulase-negative staphylococci; GNB, Gram-negative bacteria; TEWL, transepidermal water loss

aureus was seen at the second and third visits.

The alcoholic gel and alcoholic pad reduced the count of enterococci significantly at the second visit (P = 0.019 and 0.021, respectively); one hour later, a non-significant increase was observed. The same changes occurred right after using other cleansers, but they were not significant. Only in the simple wipe, the decrease continued until the third visit, though this was not significant (Figure 1e).

In terms of yeasts count (Figure 1f), a decrease occurred at the second and third visits after using the alcoholic gel, alcoholic pad, and simple wipe products. Moreover, in the group of antibacterial wipes, the yeasts count decreased initially but then increased to more than the baseline one hour after usage.

The mean TEWL increased immediately after using all the products compared to before using them (Figure 1g). This growth at the second visit was not significant in the alcoholic gel group. In the case of the alcoholic pad, antibacterial, and simple wipes, the P-values were recorded at 0.001, 0.01, and 0.001, respectively.

As Figure 1h demonstrates, all the products affected the pH of the dorsal aspect of the hand. In the alcoholic gel group, the pH was significantly decreased at the second and third visits (P = 0.01 and 0.008, respectively). At the second visit, a significant reduction in dorsal skin pH was observed in the alcoholic pad, antibacterial, and simple wipes (P = 0.022, 0.003, and 0.022, respectively).

Regarding the palmar pH (Figure 1i), an impressive reduction was observed right after using alcoholic gel and one hour after intervention (P = 0.01 and 0.003, respectively). No significant change of the palmar pH was observed in the alcoholic pad and simple wipe groups in the following visits. The antibacterial wipe reduced the palmar pH both at the second and third visits, but the changes were not significant.

In all groups, the products significantly increased stratum corneum hydration (P = 0.001, 0.011, 0.001, and 0.008 in alcoholic gel, alcoholic pad, antibacterial wipe, and simple wipe groups, respectively) (Figure 1j). However, stratum corneum hydration returned to the baseline level one hour later.

Based on ANOVA (Table 1), there were only significant differences in TEWL, dorsal and palmar pH at the first and second visits (P = 0.03, 0.001, and 0.001, respectively). Similarly, a significant difference was observed in the amount of *S. aureus* and dorsal pH at the first and third visits (P = 0.040 and 0.043, respectively).

The outcome of TEWL indicated a difference in at least two of the four studied groups. In the next step, the P-values of the pairwise comparison of products showed a significant difference between alcoholic gel and simple wipe at the first and second visits (P = 0.01) (Table 2).

Comparison of the dorsal pH by the ANOVA test

Table 1. Efficacy comparison (ANOVA test) of four studied products on skin microbiota and biometric parameters at visits 1 vs. 2 and 1 vs. 3

Variable	Change visit (%)									
	1 vs. 2					1 vs. 3				
	Alcoholic gel	Alcoholic pad	Antibacterial wipe	Simple wipe	*P**	Alcoholic gel	Alcoholic pad	Antibacterial wipe	Simple wipe	*P***
TAMC[a]	-69.06	-68.47	-3.06	308.12	0.32	-21.94	82.24	5.81	71	0.591
Yeasts	-12.96	-34.06	164.59	111.19	0.439	-6.3	-36.61	331.05	345.72	0.179
CoNS[b]	-53.29	-43.41	-46.3	85.96	0.322	59.39	427.37	189.33	-26.09	0.065
GNB[c]	-15.16	144.27	-11.35	501.29	0.398	717.86	305.95	90.95	358.8	0.514
S. aureus[d]	-26	86.3	4.54	1011.54	0.396	-1.11	667.97	86.36	-13.63	0.040
Enterococcus	-31.95	-86.61	14.13	22.05	0.21	225.79	258.55	92.99	-1396	0.485
TEWL[e]	33.62	53.89	98.62	113.99	0.03	33.62	35.45	4.15	3.13	0.56
Dorsal pH	-12.81	-3.42	-4.15	-2.71	0.001	-8.81	-4.1	-2.69	1	0.043
Palmar pH	-12.22	-0.5	-0.66	1.38	0.001	-6.40	-0.5	-2.43	0.08	0.065
Hydration	85.91	49.68	121.02	161.11	0.251	9.2	9.61	36.98	7.91	0.658

[a] Total aerobic microbial count
[b] Coagulase-negative staphylococci
[c] Gram-negative bacteria
[d] *Staphylococcus aureus*
[e] Transepidermal water loss
* The difference between first and second visits
** The difference between first and third visits

Table 2. The pairwise comparison of products for variables with significant changes at visits 1 vs. 2 and 1 vs. 3 according to ANOVA test

	Alcoholic gel/ Antibacterial wipe	Alcoholic pad/ Antibacterial wipe	Alcoholic gel/ Alcoholic pad	Alcoholic gel/ Simple wipe	Alcoholic pad/ Simple wipe	Antibacterial wipe/ Simple wipe
S. aureus[a] change *P*-value	1 vs. 2: -	1 vs. 2: -	1 vs. 2: -	1 vs. 2: -	1 vs. 2: -	1 vs. 2: -
	1 vs. 3: -	1 vs. 3: -	1 vs. 3: -	1 vs. 3: -	1 vs. 3: -	1 vs. 3: 0.044
TEWL change *P*-value	1 vs. 2: -	1 vs. 2: -	1 vs. 2: -	1 vs. 2: 0.01	1 vs. 2: -	1 vs. 2: -
	1 vs. 3: -	1 vs. 3: -	1 vs. 3: -	1 vs. 3: -	1 vs. 3: -	1 vs. 3: -
Dorsal pH change *P*-value	1 vs. 2: 0.003	1 vs. 2: -	1 vs. 2: 0.001	1 vs. 2: 0.01	1 vs. 2: -	1 vs. 2: -
	1 vs. 3: -	1 vs. 3: -	1 vs. 3: 0.046	1 vs. 3: 0.046	1 vs. 3: -	1 vs. 3: -
Palmar pH change *P*-value	1 vs. 2: 0.01	1 vs. 2: -	1 vs. 2: 0.01	1 vs. 2: 0.01	1 vs. 2: -	1 vs. 2: -
	1 vs. 3: -	1 vs. 3: -	1 vs. 3: -	1 vs. 3: -	1 vs. 3: -	1 vs. 3: -

[a] *Staphylococcus aureus*

indicated a significant difference(s) between the four studied products. In the pairwise comparison, at the second visit, there were significant differences between alcoholic gel and antibacterial wipe ($P = 0.003$), alcoholic gel and alcoholic pad ($P = 0.001$), as well as alcoholic gel and simple wipe ($P = 0.01$). At the last visit, the differences between alcoholic gel and alcoholic pad and the pair of alcoholic gel as well as simple wipe were obvious. The measured significant P-values were 0.046 for both cases (Table 2).

In addition, the palmar pH indicated a significant difference in at least three of the four studied products ($P = 0.001$; Table 1). The pairwise comparison demonstrated significant differences between alcoholic gel and antibacterial wipe, alcoholic gel and alcoholic pad, as well as alcoholic gel and simple wipe, where all P-values were 0.01 (Table 2).

According to Table 1, by comparing the first and third visits using the ANOVA test, there were significant differences in the count of *S. aureus* ($P = 0.040$). This significant P-value indicated a difference in at least one case between all the groups. In Table 2, the pairwise comparison of the products in terms of *S. aureus* represented a significant difference between the antibacterial wipe and simple wipe ($P = 0.044$).

DISCUSSION

Waterless cleansers are extensively used in places like offices, clinics, airplanes, restaurants, etc., owing to their simple, easy, and rapid effects. The important factor in the effectiveness of products is the maintenance of the epidermal barrier function [8]. This barrier interferes between skin microbiota and the acidity system to prevent the invasion of pathogens [6].

The skin microbiota consists of components such as bacteria, fungi, and viruses, which are colonized on the skin's superficial layers and under its cells [12,13]. Coagulase-negative staphylococci (including *S. epidermidis*) and coryneform bacteria have been recognized as skin-resident microbiota [14]. Indeed, to enhance immune homeostasis, the resident microbiota offers protective effects against infection from transient pathogenic microorganisms [15]. This protection is due to its microbial antagonist role in residing on epithelial surfaces [13]. Unlike this group, the transient species are usually removed by routine hand hygiene and do not usually multiply on the skin unless in cases of immune deficiency [7,13]. *S. aureus*, enterococci, and Gram-negative bacilli are considered transient pathogenic microorganisms [13].

The epidermal barrier is also composed of epithelial cells. Important biometric parameters characterize the condition of this barrier, including TEWL, hydration, and pH of the skin. Indeed, the skin's biometric parameters and environmental factors shape the microbiota and its interaction with host health [8,15]. Few studies showed the effect of staphylococci on the reduction of the TEWL value, which significantly improved the skin barrier function. In addition, low skin pH is suitable for the growth of *S. epidermidis* and other resident microbiota, while high pH is suitable for the invasion of transient microbiota such as *S. aureus* and other pathogens. In fact, the microbiota affects the skin condition and vice versa. Therefore, all these factors must be considered in parallel with each other [16-18].

Ihuma *et al.* (2013) reported that three medicated

soaps potentially reduced the number of skin resident microbiota among office workers, which might prepare the field to replace the transient microbiota [19]. Therefore, we aimed to study the efficacy of different hand cleansers to alter the composition of the skin microorganism community. In addition, due to the limited contamination among office workers and the lack of a need for strong antiseptics, we were capable of performing a targeted comparison of the different fast-acting products. Hence, in addition to hand hygiene, their role in maintaining the epidermal barrier homeostasis was also considered.

In this study, the normalization of most variables one hour after using cleansers indicated the preservation of skin integrity (Figure 1). As shown in our results about the effectiveness of cleansers on the reduction of TAMC, GNB, yeasts, and enterococci, there were no significant differences between all the studied cleansers. In most cases, the results obtained from one hour after using products indicated their short-term and transient but fast effect on reduction of the skin microbial and fungal load, which may change based on using cleansers frequently. Therefore, we can use all of them in cases where we do not have access to soap and warm water. Furthermore, we can use them at the office after touching administrative equipment to control our health in a better way. Another important issue is serving the simple wipe in many airlines in the world for cleaning or freshening, reducing the count of microorganisms before consuming in-flight meals. However, in the case of *S. aureus*, there was a significant difference at the first and third visits between antibacterial and simple wipes, which could be due to the lasting effect of benzalkonium chloride after one hour (Figure 1d). This issue was mentioned in a study that demonstrated benzalkonium chloride's persistent antimicrobial activity for a prolonged period against *S. aureus* [20].

Regarding the importance of CoNS count as part of the skin's resident microbiota, after the comparison of the four cleansers, there was no significant difference between them. Still, it is interesting to remind that the CoNS count in the alcoholic pad group approximately returned to its initial count one hour later, indicating no long-term effect. The most important CoNS is *S. epidermidis,* which is recognized as the resident microbiota preventing the growth of *S. aureus*. Environmental factors, including the use of antiseptics, can affect the skin microbiota balance and change the resident microbiota abundance, leading to infection [6,18]. Our results indicated that, in comparison between all cleansers, alcoholic pads had a less negative effect on resident microbiota; therefore, we can recommend them for long-term usage of office workers such as clinic receptionists and administrative center employees.

According to the fact that TEWL has been considered a valid determinant of epidermal damage [21], our results demonstrated that none of the four studied products in the short-term use had a destructive effect on TEWL, which eventually decreased and returned to the value at the first visit. Right after the intervention, a provisional increase of TEWL was observed in the simple wipe compared with alcoholic gel. This difference could be due to the moisturizing effect of its internal emulsion as well as the longer rubbing effect of simple wipes during usage compared to alcoholic products, which evaporated faster.

The unchanged TEWL after using alcoholic gel is consistent with previous studies conducted by Hourben *et al.* and Kramer *et al.* [22,23]. Hourben *et al.* (2006) indicated that none of the alcoholic gels altered TEWL one day after usage. From this point of view, the gels containing glycerin and 70% ethanol were preferred and close to our alcoholic gel cleanser containing 70% ethanol and lanolin emollient. In the Kramer *et al.* study, the participants were requested to use alcoholic gels for 1 and 2 weeks, and the TEWL did not change significantly [23].

It has been demonstrated that the important point to tolerate topical hygienic preparations is their effect on the acidity of hands [24]. In terms of pH, the alcoholic gel reduced the pH value in both the dorsal and palmar aspects of the hand compared with the other products. This reduction could be due to the influential effect of alcohol in the solubilization and disruption of natural moisturizer factors, sebum, and sweat gland excretion [25]. Also, the pH reduction obviously explains the significant decrease of microbial parameters. However, the pH was not less than 5, and the skin could tolerate this acidity.

Hourben *et al.* (2006) indicated that alcoholic gels reduced the skin pH, which is close to our

results [22]. The alteration in the skin pH affects the pathogenicity of microbiota, which is recognized as a regulating factor for the integrity of the barrier function [24].

In the present study, there was no difference between the products regarding hydration. Since hydration in all the groups returned to its initial rate after one hour, there is no perturbing issue. The significantly higher amount of hydration immediately after using hand cleansers can be due to their excipient ingredients such as olive oil in the antibacterial wipe, lanolin in alcoholic gel, and an emollient agent in the simple wipe.

From an economic point of view, all pads (alcoholic, antibacterial, and simple wipe) have the same price per box in the market, whereas alcoholic gels have higher prices. Regarding the content of each box/bottle, an alcoholic pad with 100 pcs per box has a reasonable price. Due to the efficacy of alcohol both against bacteria and viruses, the usage of these products would be affordable, safe for the skin, and show fast and effective results.

One limitation of this study is the lack of long-term assessment of hand cleansers. Therefore, evaluation of repeated cleaning per day and usage over a week is suggested. Another limitation is the lack of assessments on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19). Unfortunately, we conducted the study before the COVID-19 pandemic commenced. However, we know that alcohol-based products (gel, solution, and pad) are effective against SARS-CoV-2.

CONCLUSION

Skin cleansing becomes a versatile part of the daily hygiene routine, and the choice of the right cleanser is important. According to our results, alcoholic gels and pads are significantly effective immediately after usage in controlling microbial parameters compared with simple and antibacterial pads. Alcohol-based products were safe and did not disrupt the skin barrier. Therefore, these kinds of cleansers are suggested as a first choice for hand hygiene among office workers when there is limited access to soap and warm water. Also, the advantage of an alcoholic pad to preserve the resident microbiota was considered.

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Conflict of Interest: None declared.

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