**ABSTRACT**

During the current Coronavirus Disease 19 (COVID-19) pandemic, regular handwashing and other hand hygiene practices as the primary infection prevention program are highly encouraged. Notwithstanding the noticeable benefits in combating viral infection, hand hygiene practice potentially promotes dysbiosis of the skin microbiome. This review aimed to summarize the possible impacts of hand hygiene practices using soap products and alcohol-based hand rubs (ABHR) on the homeostasis of human skin microbiota. We discuss recent findings to provide evidence of the effectiveness of hand hygiene practices in viral infection control, the role of the skin microbiome in human health and the effects of hand hygiene practice on the homeostasis of the skin microbiome. Finally, this review also suggests improving research and practice of hand hygiene in response to the ongoing COVID-19 pandemic.

**Keywords:** COVID-19-related hygiene, hand wash, hand sanitizer, cutaneous microbiome, dysbiosis.


**INTRODUCTION**

The World Health Organization (WHO) declared the Coronavirus Disease 19 (COVID-19) that is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, as a global pandemic on March 12th, 2020. The virus is highly transmissible through droplets by coughing or sneezing from an infected individual. Transmission may also occur if a person touches a surface or object with the virus on it and then touches their mouth, nose, or possibly their eyes. Special attention and systematic efforts to prevent or reduce the possibility of viral transmission should be implemented. The most common recommendations are wearing masks, keeping a safe distance from others, and washing hands regularly. WHO has emphasized the importance of regular handwashing using soap and water or alcohol-based hand rubs for at least 20 seconds if hands are not visibly dirty or 40-60 seconds if visibly dirty. 

**Evidence of hand hygiene effectiveness in viral infection control**

Some of the main WHO guideline’s recommendations for personal hygiene to prevent and control the spread of COVID-19 are frequent hand washing and sanitizing with Alcohol-Based Hand Rub (ABHR), containing at least 60% ethanol. In addition, regular disinfection of fomites or commonly touched surfaces such as doorknobs, table and computer laptops, kitchen counters, bathroom’s sinks and toilets are also encouraged. Regular handwashing and good hygiene habits have been associated with a reduced risk of influenza transmission during the current COVID-19 pandemic.
The determinant factors in hygiene behavior are complex. They include demographic factors, knowledge and infrastructures of washing facilities. One of the most substantial endorsements for hand hygiene compliance is soap or hand sanitizer availability. Providing the facilities at schools and offices is recognized as the most potent factor in enhancing hand hygiene compliance and reducing the risk of viral infection. Practicing proper handwashing using the WHO method and emphasizing the importance and knowledge of hand hygiene could efficiently reduce the potential of handwashing with water and soap, the antiseptic effects of alcohol-based products on pathogens, and the recent scarcity of hand sanitizers sold in public during the COVID-19 pandemic.

Hand hygiene compliance is another critical factor that contributes to the prevention of infectious diseases. The use of various hand sanitizer products has been widely implemented in public. Hand sanitizer can also help to reduce viral transmission and is recommended to be applied before touching the facial area. WHO-formulated hand disinfectant composed of 75% (vol/vol) 2-propanol, 1.45% (vol/vol) glycerol, and 0.125% (vol/vol) hydrogen peroxide has been proven to reduce viral titers within 30 seconds by using a quantitative suspension test. An active virucidal agent is also available in ABHR and hand sanitizers containing 60-90% of ethyl alcohol or isopropyl-alcohol solutions because they inactivate almost all lipophilic viruses. Other advantages of using hand sanitizer are that it is lightweight, instant and widely accessible. Conventional handwashing practices are more encouraged to control the spread of viral infection by considering the similar potential of handwashing with water and soap, the antiseptic effects of alcohol-based products on pathogens, and the recent scarcity of hand sanitizers sold in public during the COVID-19 pandemic.

Hand washing instead of washing has been shown to be equally efficacious. Wiping hands with a wet towel soaked in water containing a mixture of 1% soap powder, 0.05% active chlorine, or 0.25% active chlorine from sodium hypochlorite can remove more than 95% of the SARS-Cov-2 virus from hands (Table 1). This study was conducted in a laboratory setting observing only one subject. Thus, future research needs to include more subjects and more handy products to test the benefit of the mentioned mixture to the greater community.

Table 1. Summary of the effectiveness of hygiene products against viral infection.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Hand Hygiene Products</th>
<th>Effectiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liu, 2017 [12]</td>
<td>General hand soap</td>
<td>The odds ratios of influenza infection significantly decreased from 0.26 to 0.029 as handwashing habits increased.</td>
</tr>
<tr>
<td>Beale, 2020 [13]</td>
<td>Antibacterial soap</td>
<td>Moderate-frequency handwashing predicted a lower personal risk of coronavirus infection. The general practice of respiratory hygiene handwashing after coughing or sneezing was associated with a lower risk of onwards transmission.</td>
</tr>
<tr>
<td>Kratzel, 2020 [14]</td>
<td>WHO hand-rub formulation</td>
<td>Viral titers of SARS-Cov-2 were reduced within the 30s to a reduction factor (RF) of &gt;3.8. Ethanol and 2-propanol were able to reduce viral titers to background levels in the 30s with RFs of between 4.8 and ≥5.9.</td>
</tr>
<tr>
<td>Ma, 2020 [15]</td>
<td>Water containing 1% (g/g) soap powder, 0.05% (g/g) active chlorine, or 0.25% (g/g) active chlorine from sodium hypochlorite.</td>
<td>SARS-Cov-2 on the palm declined by 98.36%, 96.62%, and 99.98% through wiping using the wet towel soaked in water containing 1% soap powder, 0.05% active chlorine from sodium hypochlorite, or 0.25% active chlorine from sodium hypochlorite, respectively.</td>
</tr>
</tbody>
</table>

Abbreviations: World Health Organisation (WHO), Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2).
Table 2. Interactions between human skin microbiota and host skin.

<table>
<thead>
<tr>
<th>References</th>
<th>Mediator / species</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>The symbiosis between host and human skin microbiome</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naik, 2012(^{30})</td>
<td><em>S. epidermidis</em></td>
<td>Colonization with this single commensal organism was sufficient to rescue IL-17A production. Under these inflammatory conditions, MyD88 signaling in hematopoietic cells was required to produce both IFN-γ and IL-17A.</td>
</tr>
<tr>
<td>Cogen, 2010(^{31})</td>
<td>PSMs γ and δ produced by <em>S. epidermidis</em> on normal human epidermal keratinocytes</td>
<td>PSMs γ and δ directly induced lipid vesicle leakage and exerted selective antimicrobial action against skin pathogens such as <em>S. aureus</em>. PSMs reduced GAS but maintained the survival of <em>S. epidermidis</em> on skin</td>
</tr>
<tr>
<td>Wang, 2017(^{32})</td>
<td>Staphylococcal lipoteichoic acid (LTA) product of <em>S. epidermidis</em></td>
<td>The skin microbiota drives SCF production in keratinocytes, which triggers the differentiation of dermal MCs. Microbiota impact on MCs by applying LTA, which is a TLR2 ligand. MC migration within the skin depends exclusively on keratinocyte-produced SCF</td>
</tr>
<tr>
<td><strong>Dysbiosis between host and human skin microbiome</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sari, 2020(^{33}) [McDowell, 2011, A novel multilocus sequence typing scheme for the opportunistic pathogen Propionibacterium acnes and characterization of type I cell surface-associated antigens]</td>
<td><em>Staphylococcus epidermidis</em> and <em>Cutibacterium acnes</em></td>
<td>High abundance of <em>S. epidermidis</em> and <em>Cutibacterium acnes</em> are identified in acne vulgaris</td>
</tr>
<tr>
<td>Kong, 2012(^{34})</td>
<td><em>S. aureus</em></td>
<td>The high abundance of <em>S. aureus</em> caused atopic disease (AD) and was associated with increased hBD-2 expression in SCC, which promotes tumor cell growth</td>
</tr>
</tbody>
</table>

Abbreviations: lipoteichoic acid (LTA), Toll-Like Receptor (TLR), phenol-soluble modulins (PSMs), Mast cells (MC), stem cell factor (SCF), Epidermolysis Bullosa Acquisita (EBA), human β-defensin-2 (hBD-2), Squamous Cell Carcinoma (SCC)

children.\(^{19,20}\) By all means, hand hygiene, albeit practiced using various methods, needs to be performed consistently and adequately in the population to reduce the risk of viral infection.

**Human skin microbiota in health and diseases**

The skin is the largest organ of the human body colonized by beneficial microorganisms and serves as a protective barrier to prevent the invasion of pathogens while providing a home to the commensal microbiota.\(^{7}\) The complex properties and function of the human skin continuously change due to various factors, not only from the environment but also from the alterations of the homeostatic balance inside the body, including psychological factors.\(^{21}\) Hence, the skin microbiome has been an attractive research area in recent years since it has a pronounced impact on the human state of health. The human skin hosts 21% of commensal microbiota.\(^{22}\) At least 19 distinct phyla are symbiotically residing on the skin, as shown by 16S rRNA gene sequencing. Among these, Actinobacteria (51.8%), Firmicutes (24.4%), Proteobacteria (16.5%), and Bacteroidetes
(6.3%) are the most common phyla found on the skin surface. On the genus level, *Corynebacterium*, *Propionibacterium* and *Staphylococcus* are of the highest abundance.5 Mainly, hair follicles and sebaceous glands provide niches that harbor these microbial communities.31,32 The microbial biodiversity between samples from the same habitat among subjects (beta diversity) is highest on the skin. In contrast, the diversity of the cutaneous microbiome within one person (alpha diversity) is intermediate compared to other parts of the body.24 The abundance of each microbiome population depends on the characteristics of the skin structure. For example, *Propionibacterium* predominates in the sebaceous sites on the face, whereas *Staphylococcus* and *Corynebacterium* spp. predominate in the moist areas in the bends of the elbows and between the toes of the feet.7 The populations of β-Proteobacteria and Flavobacteria prefer to reside on dry sites.5 The hands have a dry environment, resulting in the large abundance of the phyla Proteobacteria and Bacteroidetes.28 The unique populations of skin microbiota encounter continual changes due to variations in environmental factors, including pH, temperature, humidity, and exposure to antimicrobial peptides and lipids.26,27 Factors influencing skin microbiota composition are climate, exposure to ultraviolet radiation, pollution, and lifestyle factors, including diet and hygiene habits.28 The significant variability of microenvironments and the variety of individual skin treatments complicate the research concerning the correlations of skin function and skin microbiota. It has been reviewed elsewhere that the relationship between skin microbiota and the skin barrier is mediated via bacterial enzymes. Microbiota produces lipases to break down surface lipids, proteases that impact corneocyte desquamation and ureases to maintain host colonization and bacterial cells in tissues.29 Moreover, symbiotically, the skin barrier also interacts with bacteria by providing nutrients depending on its microenvironment (sebaceous, moist, and dry) and regulating bacterial balance via β-defensin production.

Advantageous interactions between skin surface microbiota and host cells were shown by staphylococcal lipoteichoic acid (LTA) inhibition of Toll-like receptor (TLR)2- and TLR3-dependent skin inflammation (Table 2).29 Commensal microbiota such as *S. epidermidis* regulate Interferon-α (IFN-α) and Interleukin-17A (IL-17A) signals to reduce and prevent inflammation synergistically.30 The LTA production by bacteria is induced by stem cell factors (SCF) released by keratinocytes, showing the interaction of commensal bacteria with host cells. These interactions result in an increased gram-positive bacterial population, which is beneficial for the skin’s health, for example, by enhancing the maturation of mast cells (MC).31 An animal study suggested that the presence of *S. epidermidis* selectively protects the skin surface from pathogens such as *S. aureus* and Group A Streptococci (GAS) by inducing the production of antimicrobial peptides such as phenol-soluble modulins (PSMs).32 Dysbiosis of skin microbiota is associated with numerous diseases such as psoriasis, an inflammatory skin disease (Table 2). It has been reviewed that the increase of Firmicutes is readily detected in psoriasis skin.33 Actinobacteria and Proteobacteria, the most prevalent and diverse phyla in healthy skin, are significantly reduced in psoriasis. The high abundance of Streptococcus and reduction of Propionibacterium have also been linked to psoriasis at the genus level. An animal study indicated that mice with reduced alpha diversity or microbial richness are susceptible to epidermolysis bullosa acquisita (EBA), a chronic skin blistering disease of autoimmune origin.33 Aligned with this study, the shift in skin microbiota composition is associated with atopic disease (AD) flares and treatment. In patients with AD, the abundance of *S. aureus* was greater during disease flares than at baseline or post-treatment and has been linked with the increase of disease severity.8 The number of *Streptomyces* is higher in normal skin compared to samples from patients with skin cancer. The overabundance of *S. aureus* is also strongly associated with increased human β-defensin-2 (hBD-2) expression in squamous cell carcinoma (SCC) which promotes tumor cell growth.36 Evidence of the impact of hygiene practice on the homeostasis of the cutaneous microbiome

As mentioned previously, a great diversity of microbiomes collectively resides on epithelial skin surfaces. The stability of individual populations of skin microbiota at the species level regardless of time intervals and external exposures has been reported.8,24 However, the skin surface is prone to the exposure of various cleansers, especially in the pandemic era when hand wash and hand sanitizer are more frequent than in normal conditions. The aim to protect from undesirable pathogenic viruses might also threaten the balance of microbiota symbiosis and host immune regulation.

The richness of bacterial species in hand swabs was significantly reduced shortly after using ABHR or washing hands with soap but gradually recovered within 1-3 days (Table 3).36 Similar results were reported after topical hand treatments with antiseptic material such as ethanol or povidone-iodine chlorhexidine.37 However, prolonged and broader application, i.e., the whole body instead of hands alone, of antibacterial soap has been reported to alter the microbial skin composition in a study in rural areas of Madagascar. With participants practicing whole-body washing with antiseptic soap for two weeks, the alteration in beta diversity was shown in a dose-dependent manner.36 The greater microbiome diversity and less accessibility of antiseptic soap in rural areas may contribute to the significance of the results.

In conclusion, it is evident that hand hygiene practice acutely altered the skin microbiota population to various extent and differed by methods. However, to date, only a few studies distinguished alteration within transient and resident microbiota. Thus, the alteration is presumably only observed in transient groups that are more susceptible to external perturbation changes than resident groups. With the present advancing methods and technology in microbiome research, it is encouraged to conduct studies that identify alteration in both transient and resident members of bacteria.

Besides the practice of handwashing, the method of drying the skin after
Table 3. Possible microbial change during hand-hygiene treatments

<table>
<thead>
<tr>
<th>The extent of alteration upon handwashing</th>
<th>Antibacterial soap</th>
<th>Non-antibacterial soap</th>
<th>Alcohol based hand rubs (ABHR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial peptides abundance</td>
<td>Peptide LL-37 significantly decreased shortly after washing and immediately recovered.44</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Species abundance</td>
<td>There was no significant change in the relative abundance of S. epidermidis in a short or more extended period after washing. A significant decrease in GAS was observed after 30 minutes of handwashing. No recovery for GAS was identified.44</td>
<td>There was no significant reduction in the abundance of S. epidermidis.44</td>
<td>The significant reduction found in S. aureus, S. marcescens, K. oxytoca, and E. coli persist for at least 30 days.14</td>
</tr>
<tr>
<td>Total count of microbiota</td>
<td>There was no significant reduction of relative total microbial abundance after 24 h.37,44</td>
<td>Reduction in total bacteria significantly in damaged hands.42</td>
<td>Significant reduction in the level of viable aerobic and anaerobic culturable bacteria immediately after product use. Recovery within &lt;24 h.37</td>
</tr>
<tr>
<td>Microbial composition</td>
<td>Immediate alteration of microbial composition. Persisted at least for two weeks.38</td>
<td>Immediate alteration of microbial composition. Recovery within 24 h-7 days.35,44</td>
<td>There was no change in microbial composition.37</td>
</tr>
<tr>
<td>Alpha diversity (species richness)</td>
<td>No alteration in richness was affected after treatment.36</td>
<td>The immediate change in OTU richness within-subject and Shannon diversity index. The change was covered immediately.37,38</td>
<td>No alteration in richness was affected after treatment.37</td>
</tr>
<tr>
<td>Beta diversity</td>
<td>Reduced diversity to greater extent than using ABHR. Recovery within 24 h.37</td>
<td>Reduced diversity is lower than antibacterial soap, which persists for at least two weeks.36</td>
<td>Reduced diversity to a greater extent compared to handwashing after seven days of treatment. Recovery &lt; 30 days.14</td>
</tr>
</tbody>
</table>

Abbreviation: Group A Streptococcus (GAS), alcohol-based hand rub (ABHR), Operational Taxonomic Unit (OTU).

washing also contributes to the amount and diversity of remaining microbiota, as shown by the results from a study comparing jet air dryer and paper towel drying methods.39 This study highlights that drying hands with a jet air dryer is the most effective method to reduce transient pathogenic bacteria than using paper towels or leaving hands wet.

Host factors and microbial factors play a role in the impact of hand sanitizers on the populations of the skin microbiome. Host factors include skin hydration, pH of the skin surface, and redness on the hand. Individuals with more hydrated skin demonstrate more significant declines in the population of culture-able bacteria after hand hygiene treatments.37 Meanwhile, the susceptibility of each microbiome taxa to the hand sanitizer treatment may also differ. The loss of minority members of the skin microbiota, such as the Staphylococcaceae and Streptococcaceae families, which are vulnerable to skin treatments, subsequently will be replaced by the most abundant taxa. Meanwhile, the family members of Propionibacteriaceae are more resilient regardless of any physical or chemical perturbations.40

It has been reviewed elsewhere that the mechanisms through which hand hygiene products can be damaging to the skin. The damage includes protein denaturation in the stratum corneum, alteration of intercellular lips, decreased cornocyte cohesion, and reduction of water-binding capacity in the stratum corneum.39 More intensified and frequent handwashing has been proposed as the culprit of cutaneous lesions and skin microbiota dysbiosis in health care workers.42,43 Specifically, in those with damaged hands, the pathogenic bacteria S. haemolyticus is found to exist in addition to more considerable numbers of S. aureus and yeast (Table 3).42 The decrease of secretion of antimicrobial peptide cathelicidin on volar forearms following soap application regardless of the type of soap was reported44 and may increase the infestation of pathogenic microbiota. The leading cause of skin microbiota dysbiosis is less well understood. It is predicted that skin microbiota dysbiosis might be caused by the depletion of the lipid barrier resulting from repeated exposure to soap or alcohol that penetrates deeper into skin layers and changes the skin microbiota.44
Perspectives on hand hygiene practices against the current COVID-19 infection
In the light of COVID-19 infection control, the WHO has recommended the following actions: placing one or several hand hygiene stations in front of all public or private buildings, performing hand hygiene using the proper techniques and according to the instructions, in particular for health workers before putting on personal protective equipment (PPE), after removing it, when changing gloves, after any contact with a patient suspected of COVID-19, and other safeguards. Nevertheless, these recommendations do not sufficiently highlight the safety of handwashing or hand hygiene practices. Instead of only promoting the knowledge and compliance of hand hygiene, the composition of hand hygiene products, the efficacy and safety assessment, and the optimum amount and frequency of product application need to be evaluated. Moreover, the indications of microbial skin alteration such as dryness and/or lesions might be added as essential criteria to consider hand hygiene formulations.

Developing the best formula for hand hygiene products with skin microbiota homeostasis is still an exciting topic to research. Products with higher alcohol concentration evaporate and dry faster than low-alcohol-containing formulas, which is associated with the log10 reduction factor. This association means that the more extended hands are in contact with alcohol, the more significant the log10 reduction of bacteria. The use of moisturizers is also recommended since they can help maintain the physical barrier function of the skin and preserve the healthy skin microbiota composition. Moisturizers also improve skin hydration by binding water to the stratum corneum, reducing redness and cracks on skin surface associated with xerosis cutis. Alternatively, the natural non-alcoholic product can also be used as hand sanitizers. For example, formulation of hand sanitizers using cellulose and Tekelan (Chromolaena odorata) leaves extract act effectively as clinical ABHR to reduce the pathogenic bacteria without adverse effects such as dry skin.

Altogether, there is currently no established evidence that either handwashing or other hand hygiene practices could insult the homeostasis of the resident microbiome on the hands in a chronic fashion. Future studies on hand hygiene practices should consider a longer time frame of observation to confirm the long-term impacts of hand hygiene practice on the skin microbiota community. The next-generation sequencing applied in microbiome research is designated to rapidly identify and analyze the microbiome biodiversity richness based on the highly conserved 16S ribosomal gene. Unfortunately, the 16S rRNA sequencing technology that the studies mentioned above cannot distinguish living or dead microbes because it assesses the total DNA in a sample. Concerning this fact, the loss of biodiversity in the microbiome on hands might be underestimated. While traditional culture methods can observe bacterial viability, this method is labor-consuming and cannot detect a broader range of unculturable bacteria colonies. During the COVID-19 pandemic, it is essential to evaluate the viability of the skin microbiome and the pathogen, in this case, SARS-Cov-2 to understand the effect of handwashing on microbiome homeostasis the efficacy of hand hygiene in preventing virus transmission. Previous studies on skin microbiome upon hand hygiene treatment mainly were conducted in a controlled laboratory setting. Therefore, it is urged to observe the hand hygiene habit in a natural community setting with accompanying laboratory analysis during a pandemic.

CONCLUSIONS
Performing hand hygiene practice is always challenging, considering the many factors such as its effectiveness in pathogen elimination and clearance while keeping a healthy residing microbial milieu. In light of the proven effectiveness of preventing viral spreading, the handwashing practice must be continuously performed. While the pandemic of COVID-19 continues, several questions remain to need to be investigated related to the effectiveness of hand hygiene practice as a SARS-CoV-2 containment attempt and its impact on the skin microbiome: 1) whether the extensive use of hand hygiene products alters long-term microbial richness and composition in different populations, 2) what is the optimal frequency of handwashing in the community level as well as clinical setting in order to not only proficiently eliminate pathogens but also prevent skin damage, 3) what is the best formula of hand hygiene products in regards to preserving healthy skin microbial populations, and lastly, 4) how to conduct studies in community and clinical settings with sufficient subjects and an appropriate time frame with optimized methods for collecting and analyzing the hand microbiome. Better understanding and formulation of hand hygiene practices will contribute to better containment of pathogens while preserving beneficial skin microbiome.

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