


Original article

The Impact of the Three Most Common Hand Cleansing Methods on the Bacterial Profile: A Randomized Clinical Trial

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ABSTRACT

Introduction: The hand harbors different species of bacteria that may play a role in the transmission of infectious diseases. Therefore, this study was conducted to determine the bacterial profile of hands and assess the efficacy of the three most common methods of hand cleansing on the reduction of that bacteria.

Materials and methods: Hand swaps were collected from 150 adults. The identity of bacteria was done by standard microbiological procedures. Each participant applied one of three selected methods of hand cleansing namely, handwashing with water and plain soap, hand rubbing with an alcohol-based sanitizer, and hand wiping with alcohol-free hand sanitizer wipes. A second swap was collected after cleansing to determine the efficacy of each method by calculating the percentage of the reduction of isolated bacteria.

Results: Most isolated bacteria were commensal flora like *Coagulase Negative Staphylococcus* (92%), and *Corynebacterium spp* (81.3%). Other pathogenic bacteria were isolated mainly, *Staphylococcus aureus* (32%), *Escherichia coli* (10%), *Pseudomonas aeruginosa* (2.6%), *Klebsiella spp* (2.6%) and *Acinetobacter spp.* (2%). The hand rubbing was more efficacy than handwashing without a statistically significant difference ($P>0.05$), and the hand wiping had lower efficacy than the other two methods with statistically significant difference ($P<0.001$).

Conclusions: The hand is a serious source of infection due to the variety of bacteria on it. These bacteria can be eliminated either by handwashing with water and plain soap or hand rubbing with an alcohol-based sanitizer. Alcohol-free hand sanitizer wipes should be used just for cleaning without disinfection due to their low efficacy as a sanitizer.

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El impacto de los tres métodos de limpieza de manos más comunes en el perfil bacteriano: un ensayo clínico aleatorizado

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RESUMEN

Introducción: La mano alberga diferentes especies de bacterias que pueden jugar un papel en la transmisión de enfermedades infecciosas. Este estudio se realizó para determinar el perfil bacteriano de las manos y evaluar la eficacia de los tres métodos más comunes de limpieza de manos en la reducción de esa bacteria.

Materiales y métodos: Se recolectaron información de las manos de 150 adultos. La identidad de las bacterias se realizó mediante procedimientos microbiológicos estándar. Cada participante aplicó uno de los tres métodos seleccionados de limpieza de manos, a saber: lavarse las manos con agua y jabón común, frotarse las manos con un desinfectante a base de alcohol y limpiarse las manos con toallitas desinfectantes para manos sin alcohol. Se recogió una segunda muestra después de la limpieza para determinar la eficacia de cada método calculando el porcentaje de reducción de bacterias aisladas.

Resultados: La mayoría de las bacterias aisladas fueron flora comensal como *Staphylococcus Coagulasa Negativo* (92%) y *Corynebacterium spp* (81,3%). Se aislaron otras bacterias patógenas principalmente, *Staphylococcus aureus* (32%), *Escherichia coli* (10%), *Pseudomonas aeruginosa* (2,6%), *Klebsiella spp* (2,6%) y *Acinetobacter spp.* (2%). Frotar las manos fue más eficaz que lavarse las manos sin una diferencia estadísticamente significativa ($P > 0,05$), y la limpieza de las manos tuvo una eficacia menor que los otros dos métodos con una diferencia estadísticamente significativa ($P < 0,001$).

Conclusiones: La mano es una fuente grave de infección debido a la variedad de bacterias que contiene. Estas bacterias pueden eliminarse lavándose las manos con agua y jabón común o frotándose las manos con un desinfectante a base de alcohol. Las toallitas desinfectantes para manos sin alcohol deben usarse solo para limpiar sin desinfectar debido a su baja eficacia como desinfectantes.

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1. INTRODUCTION

The skin is one of the most human-associated microbial habitat; it has a huge number of bacteria that have an important impact on our health, about 1×10^7 bacteria/cm² of the skin surface [1]. Exposure to external conditions and the effect of intrinsic factors make the skin a dynamic environment alter continuously its bacterial profile.

In 1847, Dr. Ignaz Semmelweis highlighted the importance of handwashing in decreasing puerperal fever cases [2]. Since then, many studies have pointed out the efficacy of handwashing in reducing other infections especially gastrointestinal and respiratory infections [3], due to the ability of handwashing in reducing the transmission by eliminating potential organisms and by washing off the dirt that could harbor some nutrients essential for the survival of these microorganisms for a longer time. World Health Organization (WHO) and the United Nations (UN) declared 15 October as the Global Handwashing Day to address the

issue of the importance of improving hand hygiene performance [4]. Despite many procedures and measures, poor hand hygiene practice has been reported in many communities including students, doctors, and nurses across the world [5]. Availability of facilities is the key issue that makes handwashing practicing more applicable and efficient, such as the availability of water and soap. Therefore, finding alternative cleansing methods characterized by ease, efficacy, and availability is an urgent need especially in countries that have a long history of endemic infections.

Alcohol-based sanitizer is the most common way that was introduced to substitute handwashing using soap and water to prevent the transmission of microorganisms and decrease the healthcare burden, a range of various concentrations and forms of delivery are available. Recent studies have found better compliance in hand cleansing after the introduction of hand rubbing as an alternative method [6]. Hand sanitizer wipes are also an alternative method of handwashing, they are available with different antiseptic ingredients, and the

most common one is benzalkonium chloride [7]. The ease of using them and their distinctive ability to clean makes many people depend on it on a large scale especially in the childhood period.

This study aimed to identify the bacterial profile of the hand and how it is affected by three common methods of hand cleansing in the community namely, handwashing with plain soap and water, hand rubbing with an alcohol-based sanitizer, and hand wiping with alcohol-free hand sanitizer wipes.

2. MATERIALS AND METHODS

2.1. STUDY DESIGN AND THE SUBJECTS

The study was a prospective randomized clinical trial, which was done among university students in Kurdistan Region-Iraq between November 2020 and March 2021. The study was committed to the blinded analysis of results to identify the bacterial profile of the hand and assess the efficacy of hand cleansing methods.

Potential participants who aged more than 18 years who approved to engage in the study were recruited. Any having acute or chronic lesion related to the skin and/ or nails were excluded.

All participants in the study approved the involvement in the study after they were informed about the aim and the protocol of the experiment, with confirmation that all data would be treated confidentially. The study was approved by the university Ethics Committee and according to Helsinki ethical principles declaration.

2.2. SAMPLES COLLECTION

The dominant hand was swept by the sterile normal saline dunked cotton swab, the sweeping began from the flexor side of the palm towards the five fingers and ended with the dorsal side. After hand cleaning a second sample was collected exactly like the first one, taking into account not to dry the hand after washing with any drier. The swabs were transported to the laboratory within two hours. At the lab, the swabs were inoculated on blood agar and MacConkey agar using standard streak plate procedure. The plates were incubated at 35°C for 24 hours. The identification was done by standard microbiological procedures, which include colony morphology, gram stain, and biochemical tests [8]. The examination of cultures and reporting the results were done without knowing which hand cleansing method was used.

2.3. HAND CLEANSING METHODS

Three methods of hand cleansing were chosen according to the popularity of their use in the community. The first method was handwashing with water and plain soap, hands were washed with soap for 20 seconds following the handwashing guidelines recommended by the Centers of Disease Control and Prevention, and then hands were rinsed thoroughly for 20 seconds under running water. The second method was hand rubbing with an alcohol-based sanitizer, the concentration of alcohol was 70% which is the most available concentration in the market, the pump of sanitizer added 2 ml in the palm of one hand that is enough to keep hands wet for 20 seconds that is the time of rubbing. The third method was hand wiping with alcohol-free hand sanitizer wipes. We chose hand wipes that contain benzalkonium chloride as antiseptic because it is the most common one in the market, and the duration of wiping was 20 seconds.

3. RESULTS

The current study included 150 adults, and the organisms which were isolated before cleaning were: *Coagulase-Negative Staphylococci (CoNS)* (92%), *Corynebacterium spp.* (81.3%), *Staphylococcus aureus* (32%), *Bacillus spp.* (23.3%), *Escherichia coli* (10%), *Enterococcus spp.* (4.6%), *Pseudomonas aeruginosa* (2.6%), *Klebsiella spp.* (2.6%), and *Acinetobacter spp* (2%), whereas 8% were sterile.

The participants were divided into three groups and each group has 50 participants. The first group washed their hands with water and plain soap, the second group applied hand rubbing with an alcohol-based sanitizer for cleaning, and the third group applied hand wiping with alcohol-free hand sanitizer wipes, the results of isolated bacteria before and after the cleansing of the three groups are shown in Figure 1, Figure 2, and Figure 3 respectively.

Table 1: The comparison between the results of hand washing with water and plain soap and hand rubbing with alcohol-based sanitizer

| Bacteria | Water and plain soap (n=50) | | Alcohol-based sanitizer (n=50) | | P value |
|-------------------------------|-----------------------------|-------------|--------------------------------|-------------|---------|
| | Before n (%) | After n (%) | Before n (%) | After n (%) | |
| CoNS | 44 (88%) | 11 (22%) | 49 (98%) | 9 (18%) | 0.1 |
| <i>Corynebacterium spp.</i> | 40 (80%) | 11 (22%) | 38 (76%) | 8 (16%) | 0.08 |
| <i>Staphylococcus aureus</i> | 16 (32%) | 2 (4%) | 17 (34%) | 0 (0%) | 0.09 |
| <i>Bacillus spp.</i> | 14 (28%) | 2 (4%) | 11 (22%) | 2 (4%) | 0.5 |
| <i>Escherichia coli</i> | 5 (10%) | 1 (2%) | 6 (12%) | 1 (2%) | 0.2 |
| <i>Enterococcus spp.</i> | 2 (4%) | 0 (0%) | 3 (6%) | 0 (0%) | 0.1 |
| <i>Pseudomonas aeruginosa</i> | 2 (4%) | 0 (0%) | 1 (2%) | 0 (0%) | 0.2 |
| <i>Klebsiella spp.</i> | 2 (4%) | 0 (0%) | 2 (4%) | 0 (0%) | 0.1 |
| <i>Acinetobacter</i> | 1 (2%) | 0 (0%) | 2 (4%) | 0 (0%) | 0.1 |

CoNS: Coagulase-negative staphylococci. P value according to one-way ANOVA was > 0.05 for all genera, which indicates no statistically significant difference between both methods

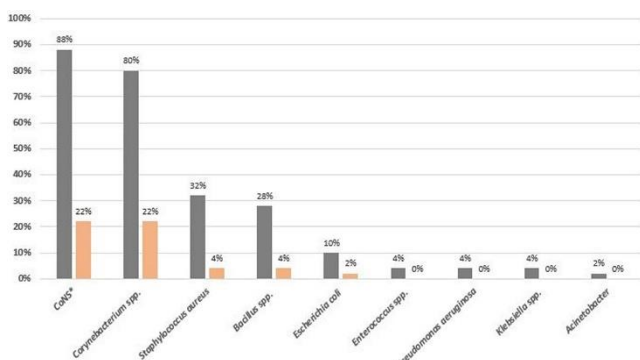


Figure 1: Distribution of isolated bacteria before and after handwashing with water and plain soap. Grey colour summarize the results prior the intervention. Orange shows the results after the intervention.

CoNS: Coagulase-negative staphylococci.

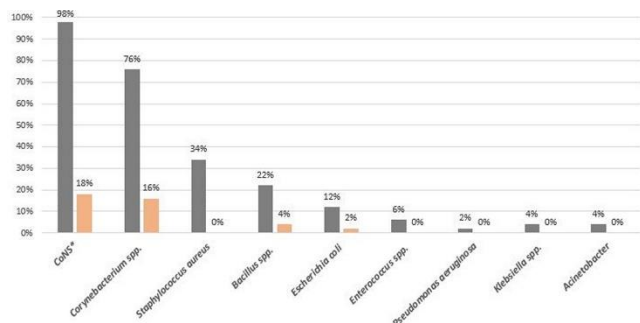


Figure 2: Distribution of isolated bacteria before and after hand rubbing with alcohol-based sanitizer. Grey colour summarize the results prior the intervention. Orange shows the results after the intervention.

CoNS: Coagulase-negative staphylococci.

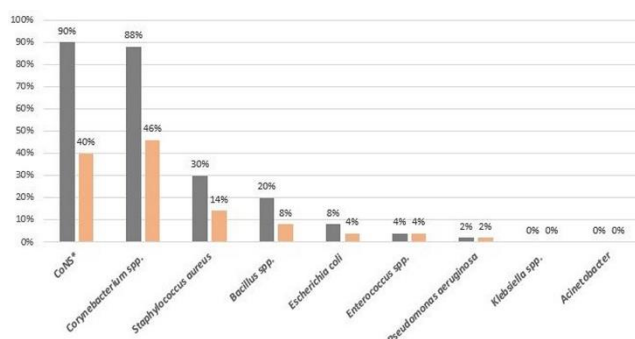


Figure 3: Distribution of isolated bacteria before and after hand wiping with alcohol-free hand sanitizer wipes. Grey colour summarize the results prior the intervention. Orange shows the results after the intervention.

CoNS: Coagulase-negative staphylococci.

Although the efficacy of alcohol-based sanitizer was higher than handwashing with water and plain soap, there was not a statistically significant difference between both methods (P > 0.05) according to one-way ANOVA analysis (Table 1). Whereas hand wiping with alcohol-free hand sanitizer wipes has considerably lower efficacy than the other two methods (handwashing and hand rubbing) with a statistically significant difference (P < 0.05) (Table 2, Table 3).

4. DISCUSSION

The current study showed that the most common bacteria on the hands were *Coagulase Negative Staphylococcus* (CoNS) (92%), and *Corynebacterium spp* (81.3%) which are considered commensal flora. However, these bacteria are opportunistic pathogens, as they inhabit the skin and mucous membranes of healthy people and turn into pathogens mainly for predisposed patients [9]. Many studies are consistent with our results [10, 11]. *S. aureus* was isolated

from 32% of participants. Although they are a part of the normal skin flora, they are the leading pathogen of skin and soft tissue infections and can cause serious infections such as endocarditis and septicemia when reaching the blood [12, 13].

chemicals [20]. This recommendation was supported by a systematic review in 2016 about the usage of hand sanitizers in settings of food preparation and suggested that handwashing with water and soap is more effective than alternative methods [21]. On the other hand, Abaza et al

Table 2: The comparison between the results of hand washing with water and plain soap and hand wiping with alcohol-free hand sanitizer wipes

| Bacteria | Water and plain soap (n=50) | | Alcohol-based sanitizer (n=50) | | P value |
|-------------------------------|--------------------------------|----------------|-----------------------------------|----------------|---------|
| | Before n (%) | After n (%) | Before n (%) | After n (%) | |
| <i>CoNS</i> | 44 (88%) | 11 (22%) | 45 (90%) | 20 (40%) | 0.001 |
| <i>Corynebacterium spp.</i> | 40 (80%) | 11 (22%) | 44 (88%) | 23 (46%) | 0.01 |
| <i>Staphylococcus aureus</i> | 16 (32%) | 2 (4%) | 15 (30%) | 7 (14%) | 0.01 |
| <i>Bacillus spp.</i> | 14 (28%) | 2 (4%) | 10 (20%) | 4 (8%) | 0.02 |
| <i>Escherichia coli</i> | 5 (10%) | 1 (2%) | 4 (8%) | 2 (4%) | 0.02 |
| <i>Enterococcus spp.</i> | 2 (4%) | 0 (0%) | 2 (4%) | 2 (4%) | 0.04 |
| <i>Pseudomonas aeruginosa</i> | 2 (4%) | 0 (0%) | 1 (2%) | 1 (2%) | 0.04 |
| <i>Klebsiella spp.</i> | 2 (4%) | 0 (0%) | 0 (0%) | 0 (0%) | - |
| <i>Acinetobacter</i> | 1 (2%) | 0 (0%) | 0 (0%) | 0 (0%) | - |

CoNS: Coagulase-negative staphylococci. P value according to one-way ANOVA was > 0.05 for all genera, which indicates no statistically significant difference between both methods

Other bacteria appeared on the participants' hands that are involved in community-acquired infection, for example, *E. coli* that have been identified as one of the most pathogens responsible for diarrhea especially in developing countries, and used as an indicator of fecal-water contamination [14]. In the current study, 10% of participants harbored *E. coli*, whereas just 2.6%, 2.6%, and 2% of participants had *Pseudomonas aeruginosa*, *Klebsiella spp.*, and *Acinetobacter spp.* respectively on their hands. These bacteria associate with an opportunistic infection that varies from localized infections of the skin to life-threatening systemic disease, as it is very easy for these bacteria to find their way to the gastrointestinal and respiratory tracts when we contact our mouths and noses [15-17]. These rates are comparatively lower than the results of Kavitha and Ray et al studies [10, 18] and higher than Nasution's study [19], these differences are due to the difference in the compliance of hygiene practices. When the study is carried out among children who have low compliance as Ray et al study, the contamination increases [18], and when the study is carried out among medical staff who have high compliance like Nasution's study, the contamination decreases [19]. This fact confirms the importance of finding easy and effective methods of hand cleansing to ensure good compliance in the community leading to decrease infectious diseases transmission and their medical and economic burden.

Centers for Disease Control and Prevention (CDC) recommended washing hands with water and soap over hand sanitizers whenever possible due to the ability of handwashing to expel a wide spectrum of pathogens and

found the results of alcohol-based hand rub are much more efficient than handwashing with water and soap [22], this due to the study was conducted in the Intensive Care Unit (ICU) where the strains of bacteria are more resistant and need stronger antiseptic than normal washing by water and soap. Alcohol has many toxic effects and it is a non-specific antimicrobial by causing clumping of cell protein especially cell membrane leading to lose their function [23].

In the current study, we found the hand rubbing with an alcohol-based sanitizer was more efficacy than handwashing with water and plain soap but without statistically significant difference. That means both methods have the same efficacy, and this result is consistent with Nasution et al study [19].

Hand sanitizer wipes are a common method of hand cleaning especially among mothers who take care of young children. All the brands of alcohol-free hand sanitizer wipes in the market have an unknown concentration of ingredients particularly the antimicrobial agents, benzalkonium chloride (BAC) is the most antimicrobial ingredient in hand sanitizer wipes. The United States Food and Drug Administration (US FDA) classified BAC as a category III antiseptic ingredient for the lack of sufficient safety data [24]. The action of BAC depends on the concentration; it is used as a preservative by a concentration ranges from 0.004 to 0.01%, while the concentration of 0.15% is considered the optimum as antimicrobial [25]. The current study showed that hand

Table 3: The comparison between the results of hand rubbing with alcohol-based sanitizer and hand wiping with alcohol-free hand sanitizer wipes

| Bacteria | Water and plain soap (n=50) | | Alcohol-based sanitizer (n=50) | | P value |
|-------------------------------|--------------------------------|----------------|-----------------------------------|----------------|---------|
| | Before n (%) | After n (%) | Before n (%) | After n (%) | |
| <i>CoNS</i> | 49 (98%) | 9 (18%) | 45 (90%) | 20 (40%) | 0.03 |
| <i>Corynebacterium spp.</i> | 38 (76%) | 8 (16%) | 44 (88%) | 23 (46%) | 0.09 |
| <i>Staphylococcus aureus</i> | 17 (34%) | 0 (0%) | 15 (30%) | 7 (14%) | 0.04 |
| <i>Bacillus spp.</i> | 11 (22%) | 2 (4%) | 10 (20%) | 4 (8%) | 0.03 |
| <i>Escherichia coli</i> | 6 (12%) | 1 (2%) | 4 (8%) | 2 (4%) | 0.03 |
| <i>Enterococcus spp.</i> | 3 (6%) | 0 (0%) | 2 (4%) | 2 (4%) | 0.001 |
| <i>Pseudomonas aeruginosa</i> | 1 (2%) | 0 (0%) | 1 (2%) | 1 (2%) | 0.001 |
| <i>Klebsiella spp.</i> | 2 (4%) | 0 (0%) | 0 (0%) | 0 (0%) | - |
| <i>Acinetobacter</i> | 2 (4%) | 0 (0%) | 0 (0%) | 0 (0%) | - |

CoNS: Coagulase-negative staphylococci. P value according to one-way ANOVA was > 0.05 for all genera, which indicates no statistically significant difference between both methods

wiping with alcohol-free hand sanitizer wipes was less efficacy than handwashing with water and plain soap and hand rubbing with alcohol sanitizer in reducing isolated bacteria of the hand, and this difference was statistically significant ($P < 0.05$), this result is consistent with Sickbert et al study [26]. Although BAC was approved as an antimicrobial in many studies [25, 27], this effect extremely depends on the proper preparation with proper concentration, which is maybe not achieved in many hand wipes industries.

The current study – to our knowledge- is the first one in Kurdistan-Iraq that evaluates the efficacy of hand wet wipes as an antiseptic agent, but it was limited to the antibacterial effect, further studies include the antiviral and antifungal activity should be conducted.

5. CONCLUSIONS

The hand harbors a variety of normal flora and pathogenic bacteria that are involved in many community-acquired infections, these organisms could be removed by handwashing with water and plain soap, and when this method is unavailable or inconvenient, hand rubbing with alcohol-based sanitizer is an active alternative method. The efficacy of alcohol-free hand wipes as antiseptic is very limited, and the usage of them should be saved just for cleaning without disinfection unless the concentration of the antiseptic ingredient is clear and consistent with the global indication.

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7. CONFLICT OF INTERESTS

The authors have no conflict of interest to declare. The authors declared that this study has received no financial support.

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