Bacteria decontamination after different hand hygiene procedures in dental assistants during patient care

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Abstract

Objectives: This study aimed to evaluate the efficacy of bacterial decontamination of different hand-hygiene procedures in dental personnel.

Materials and Methods: The crossover design experiment was carried out in 12 dental assistants using 4 hand-hygiene methods, i.e., (A) hand washing with un-medicated soaps, (B) hand washing with antiseptic soaps, (C) hand washing with un-medicated soaps plus alcohol-based hand rub, and (D) only alcohol-based hand rub. The samples were collected, using sterile cotton swabs, from palms and fingertips of the participants after chair-side working in the dental clinic as baseline data. After that they washed their hands as instructed by the guideline of each method in random order. After hand washing, the samples were again collected as previously described. The swab samples were immersed in D/E Neutralizing broth and the amount of bacteria were determined by plate counting method. In addition, the temperature, relative humidity, and carbon dioxide levels in the dental clinic were measured by Q-Trak Plus IAQ Monitor Model 8554.

Results: This study found that the amount of bacteria on the fingertips was more than on the palms. All 4 hand hygiene methods could significantly reduce the bacteria on the hands (p <0.05). On the palms, 52 percent bacterial reduction was shown by method A and it showed a significant difference when compared to method B, C, and D which showed 100 percent reduction. On the fingertips, the percent bacterial reduction by method A to D was 16, 77, 80, and 80, respectively. Additional data from the questionnaires indicated that approximately 50 % of the dental assistants prefer method C less to other methods because it required 2 steps of washing. Regarding the air quality in the dental clinic, the average values of the temperature was 23.6°C, the carbon dioxide level was 736 ppm, and those were in accordance with ASHRAE suggestions. However, the relative humidity was 71.2 % which was higher than the recommendation.

Conclusion: Our study highlights the efficacy of ABHR gel formulation which is a valid and acceptable hand hygiene method that can be routinely use and may increase the compliance of hand hygiene in a dental setting.

Key Words: alcohol-based hand rub, bacterial decontamination, dental assistants, hand hygiene, hand washing, infection control


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Introduction

Infection in hospital setting or nosocomial infection is one of the major public health problems causing several impacts, i.e., longer duration of care, increase morbidity and mortality, more cost. The spread of microbes in hospitals can occur via direct contact with patients, biologic fluids, and indirect contact with contaminated materials, i.e., medical equipment or devices, air, and environments. Health care workers (HCWs) are the most important vehicle for the transmission of pathogens, especially antimicrobial-resistant strains, via contaminated hands in clinical setting mostly during patient care. Hand hygiene is therefore the critical procedure to prevent the spread of infection and reduce the incidence of nosocomial infection and health care-associated infections. Several studies have shown that proper hand washing with soap-unmedicated or medicated, and alcohol-based hand rub (ABHR) can significantly reduce the contaminated bacteria on hand skin. However, compliance with hand washing by HCWs has been unacceptably low, generally less than 50%, and in many cases the hand washing procedure is inadequate. Reasons for low compliance are vary from place to place; including insufficient facilities such as sink, water, soap, paper towels, unaware of the importance, lack of information and training, lack of time, interruptions of regular care procedures, skin dryness, irritations, and allergies.

In dental settings, aerosols and droplets are produced during many dental procedures. Dental healthcare personnel, i.e., dentists, dental assistants, are generally infected by microorganisms from the oral cavity via direct contact with lesions, saliva, or blood, indirect contact via contaminated intermediate objects, or airborne transmission. Frequently patients appear clinically well and are unaware that they have infected with a particular pathogen. For example, they may be carriers of tuberculosis, respiratory tract viruses, blood-borne viruses such as hepatitis B viruses, HIV. Additional to glove wearing, hand hygiene is routinely used as a primary measure for reducing the contaminated microorganisms on hands of dental health care workers (DHCW). However, study on hand hygiene attitudes, compliance, and efficacy in dental healthcare workers are still limited.

Alcohol-based hand rub is introduced as easy access hand disinfection and it can overcome aforementioned obstacles in the hand washing with soap and water. Several studies have shown that hand rubbing with alcohol based is more efficient than hand washing with un-medicated soap and at least as effective as with medicated soap. Thus the compliance of hand hygiene among HCWs has improved due to the increased uses of ABHR. However, some practitioners still reluctance to accept ABHR due to the lack of confidence about its efficacy. Evaluation of the efficacy of different hand hygiene techniques in clinical and dental situation is still very few.

This study therefore aimed to investigate the efficacy of four different hand hygiene procedures in dental settings.

Materials and Methods

This study was carried out during February to April 2015 at the Periodontic clinic of Faculty of Dentistry, Mahidol University, Bangkok, Thailand. The study protocol was approved by the Committee on Human Rights Related to Human Experimentation, Faculty of Dentistry/Faculty of Pharmacy, Mahidol University, Bangkok (COA.No.MU-DT/PY-IRB 2015/003.3001).

Subjects

Dental assistants, working in the Periodontic clinic were recruited in this study. They aged between 20-40 years old and had working experience not less than 1 year. After been informed about the research protocol, those
who interested in the study were asked to sign the consent form. The participants who had wound or injury on the hand, or had an allergic reaction to the hand washing products were excluded from the study. Total of 12 dental assistants were participated in this study. They were interviewed using the questionnaires which comprised of the basic demographic data such as sex, age, working experience, the characteristics of hands and nails, history of allergic to any hand washing products. After hand-washing they were asked to evaluate their satisfaction to the test procedures.

**Study design**

This study was a crossover design which aimed to evaluate the efficacy of four hand-hygiene procedures to decontaminate the bacteria on the hands of dental assistants in everyday practice. Each subject performed hand hygiene procedures in random order. The samples were collected from the palm and the fingertips of the dominant hand before and immediately after hand-hygiene procedures. Each hand-hygiene procedure was performed 1 week apart. Therefore, 48 sets of 96 samples was obtained totally.

On the day of sample collection, dental assistants were allowed to work in the dental clinic for at least 1 hour. After a dental treatment, the samples were collected immediately after removed the gloves. Then each dental assistant was assigned to perform either one of 4 hand hygiene procedures in random order. Hand hygiene procedures were as follows:

A) Hand washing with unmedicated soap (Kleenex, USA) 10 ml,
B) Hand washing with antiseptic liquid soap containing 4% chlorhexidine gluconate (Hydrex, England) 10 ml,
C) Hand washing with un-medicated soap (Kleenex, USA) 10 ml, followed by alcohol-based hand-rub gel containing 60-70% ethanol and 0.1-0.3% triclosan (Virulex, Thailand) 10 ml,
D) Hand rubbing with alcohol based gel (Virulex, Thailand) 10 ml

The guideline of hand washing was comprised of 6 steps as follows: 1) palm to palm, 2) palms over dorsums, 3) fingers interlace palm to palm, 4) back of fingers to opposing palms, 5) rotational rubbing of both thumbs, 6) rotational rubbing of both palms. The washing time was approximately 1 minute.

After washing the hands, the samples were again collected 1 min later. In addition, the hands and nails of subjects were observed and recorded. Therefore, 4 records were obtained from each subject.

**Sample collection for bacterial evaluation**

The samples were collected from the palm and fingers of the dominant hand of each dental assistant before and after each hand-washing using the sterile cotton swabs. The swab was used to wipe at the center of the palm, approximately 5 x 5 square centimeters and then placed in 2 ml D/E Neutralizing broth (Difco & BBL, USA). Another swab was used to wipe the tips of forefinger, middle finger, and ring finger; and then placed in 2 ml D/E Neutralizing broth. The total area was approximately 3 x 4 square centimeters. All samples were sent to the microbiology laboratory, Faculty of Dentistry, Mahidol University for bacterial culture.

**Bacterial culture and enumeration**

The amount of bacteria in each sample was determined by plate counting method. Upon laboratory arrival, each swab sample in D/E Neutralizing broth was vortexed to disperse bacteria from the swab. The broth sample was then serially diluted ten-fold in D/E Neutralizing broth for 5 dilutions. An aliquot of 100 µl from each dilution was plated on Plate count agar (Difco & BBL, USA) in duplicate and
incubated at 37°C for 48 h. After incubation period, the number of bacteria colony in each sample was calculated and expressed as CFU (colony forming unit) per ml of sample and per square centimeters.

**Collection of air samples**

The air quality in the clinic was measured during the study. The schedule was on Tuesday and Thursday from 10.00 to 11.30 a.m. for 6 consecutive weeks. The temperature, relative humidity and carbon dioxide levels were measured directly by Q-Trak Plus IAQ Monitor Model 8554. - Direct reading instrument Q-Trak Plus IAQ Monitor Model 8554 (TSI, USA)

**Statistical analysis**

Basic demographic data were presented as the percentage, mean, standard deviation, maximum and minimum values. Bacterial counts from each sample as CFU were transformed into Log 10. The following statistical methods were used for data analysis by SPSS version 18.0. Komogorov-Smirnov test was used to testing the normality distribution of the data. For calculating differences of bacteria quantity before and after each hand washing procedure, the student paired-t test was used. Percent reduction of bacteria quantity after each hand washing procedure was calculated and used to compare statistical differences among the test groups by one way ANOVA. Multiple comparison LSD was further used to indicate the significant difference between the methods. A level of significance was considered at $\alpha = 0.05$.

**Results**

A total of 12 dental assistants was participated in this study; 11 females (91.7%) and 1 male (8.3%). Their ages ranged from 20-34 years old (mean = 24.8 ± 4.2). They had been working as dental assistants for 1-13 years (mean = 3.3 ± 3.6). All subjects are right-handed. They did not wear nail color. On the day of sample collection, the hands and nails of subjects were observed and recorded. The long finger nails were found 36 out of 48 times of record (75%). They all wore gloves throughout the treatment of a patient. They had to randomly perform either one of the 4 hand hygiene procedures after working chair-side. After they did all 4 hand hygiene procedures, they were asked to evaluate which was the most preferred method. All dental assistants satisfied with hand-washing method A) with un-medicated soap, B) with antiseptic liquid soap, and D) alcohol based hand rub. Seven out of 12 subjects (58.3%) disliked hand-washing method C) with un-medicated soap followed by alcohol based hand rub because they thought that it was not practical.

Regarding the cost of each hand-washing method, it was calculated based on the product price. By the quantity of 1,000 ml, the price of un-medicated soap was 275 bahts, antiseptic soap was 300 bahts, and alcohol-based hand rub was 390 bahts. Therefore, the cost of method A, B, C, and D, was 2.75, 3.00, 6.65, and 3.9 bahts per time of washing, respectively.

Indoor air quality (IAQ) of the clinical zone was measured randomly 2 times a week for 6 consecutive weeks. The mean temperature was 23.6°C (SD = 0.20), the mean relative humidity was 71.2% (SD = 0.57), and the mean carbon dioxide level was 736 ppm (SD = 7.27).

**Bacterial quantity before and after different hand-hygiene procedures**

To evaluate the efficacy of each hand-hygiene method, the amount of bacteria on palms and fingernails was compared before and after hand washing. The total bacterial counts on the hands of the dental assistants ranged from 0.4 x 10$^1$ to 2.1 x 10$^2$ CFU/cm$^2$, with the mean of 4.18 x 10$^1$ CFU/cm$^2$. The total bacterial counts
on the fingers of the dental assistants ranged from $1.5 \times 10^1$ to $4.8 \times 10^5$ CFU/cm$^2$, with the mean of $1.93 \times 10^5$ CFU/cm$^2$.

The log reduction of bacteria in the samples from the palms and fingers after different hand washing methods was shown in Figure 1 and 2. After hand washing, it was found that all procedures could significantly

![Box plot showing the log reduction of bacteria in the samples from the palms after 4 different hand-hygiene procedures. A) Hand washing with unmedicated soap, B) Hand washing with antiseptic soap, C) Hand washing with unmedicated soap and alcohol-based hand rub, D) Alcohol-based hand rub.](image1)

![Box plot showing the log reduction of bacteria in the samples from the fingers after 4 different hand-hygiene procedures. A) Hand washing with unmedicated soap, B) Hand washing with antiseptic soap, C) Hand washing with unmedicated soap and alcohol-based hand rub, D) Alcohol-based hand rub.](image2)
reduce the amount of bacteria (p-value ≤ 0.05) on palms and on fingers as shown in Table 1 and Table 2, respectively. The bacteria could not be detected in the palm samples after using hand washing method B, C, and D (Table 1).

Comparison of efficacy for bacterial decontamination of 4 hand-hygiene methods

The efficacy of the hand-hygiene methods was compared using the percent log bacterial reduction on the palms and the fingers after hand washing. On the palms, it was found that the percent reduction was 52 for method A, and 100 for method B, C and D. On the fingers, the percent reduction was 16, 77, 80, and 80 for method A, B, C and D, respectively, as shown in Figure 3. Statistical analyses were shown that the bacteria reduction by the method A was significantly lower than method B, C, and D (p-value ≤ 0.05), as shown in Table 3.

Discussion

Bacterial aerosols and droplets produced during dental procedures are sources of disease transmission to dental health care workers and patients. The personal protective measures for DHWCW to reduce contamination include wearing masks, gloves and gown as well as hand hygiene procedures. This study aimed to compare the efficacy of 4 hand hygiene procedures in reducing bacteria quantity on the hands after dental treatment. The study was conducted in the Periodontic clinic as aerosolized contaminants commonly generated during dental procedures, i.e., ultrasonic scaling and root planning. We performed the study in dental assistants, who are considered routinely at an increased risk of cross-infection while assisting

Table 1  The quantity of bacteria (log CFU/ml) in samples from palms before and after 4 different hand-hygiene procedures (A-D).

<table>
<thead>
<tr>
<th>Hand hygiene procedures#</th>
<th>Log CFU/ml</th>
<th>Log Reduction</th>
<th>Percent Log Reduction</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>2.68 ± 0.30</td>
<td>1.30 ± 0.70</td>
<td>1.38 ± 0.45</td>
<td>52</td>
</tr>
<tr>
<td>B</td>
<td>2.49 ± 0.28</td>
<td>Not detected</td>
<td>2.49 ± 0.28</td>
<td>100</td>
</tr>
<tr>
<td>C</td>
<td>2.56 ± 0.36</td>
<td>Not detected</td>
<td>2.56 ± 0.36</td>
<td>100</td>
</tr>
<tr>
<td>D</td>
<td>2.50 ± 0.59</td>
<td>Not detected</td>
<td>2.50 ± 0.59</td>
<td>100</td>
</tr>
</tbody>
</table>

Note

# A) Hand washing with unmedicated soap, B) Hand washing with antiseptic soap, C) Hand washing with unmedicated soap and alcohol-based hand rub, D) Alcohol-based hand rub.

* Significant at p-value ≤ 0.05, a = paired-t test

Table 2  The quantity of bacteria (log CFU/ml) in samples from fingers before and after 4 different hand-hygiene procedures.

<table>
<thead>
<tr>
<th>Hand hygiene procedures#</th>
<th>Log CFU/ml</th>
<th>Log Reduction</th>
<th>Percent Log Reduction</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>5.46 ± 1.31</td>
<td>4.56 ± 1.40</td>
<td>0.90 ± 0.23</td>
<td>16</td>
</tr>
<tr>
<td>B</td>
<td>5.39 ± 1.61</td>
<td>1.26 ± 1.11</td>
<td>4.13 ± 1.16</td>
<td>77</td>
</tr>
<tr>
<td>C</td>
<td>5.27 ± 1.50</td>
<td>1.06 ± 1.16</td>
<td>4.21 ± 1.23</td>
<td>80</td>
</tr>
<tr>
<td>D</td>
<td>5.25 ± 1.56</td>
<td>1.07 ± 1.15</td>
<td>4.18 ± 1.34</td>
<td>80</td>
</tr>
</tbody>
</table>

Note

# A) Hand washing with unmedicated soap, B) Hand washing with antiseptic soap, C) Hand washing with unmedicated soap and alcohol-based hand rub, D) Alcohol-based hand rub.

* Significant at p-value ≤ 0.05, a = paired-t test
dentists treating the patients. We designed the study similar to everyday practice by collecting samples after they routinely assist treating patients for at least 2 cases to allow hand contamination. Another previous study has conducted the experiments by contaminating the hands with the bacteria before hand-washing which may not safe and unacceptable to volunteers. After finish assisting a patient, the samples for bacteria detection were collected from the palms and fingers. One set included the samples collected immediately after glove removed, and immediately after hand-washing. We used sterile cotton swabs for sample collection because it is practical for particular areas that have been reported the finding of bacteria after hand-washing; e.g., the nails. In addition, it enables the laboratory...

Figure 3 Comparison of percent log bacterial reduction on the palms and the fingers after using 4 hand hygiene procedures.
A) Hand washing with unmedicated soap,
B) Hand washing with antiseptic soap,
C) Hand washing with unmedicated soap and alcohol-based hand rub,
D) Alcohol-based hand rub.

Table 3 Comparison of the efficacy of 4 different hand hygiene procedures for bacterial reduction on the palms and the fingers.

<table>
<thead>
<tr>
<th></th>
<th>Log reduction</th>
<th>p-value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Difference&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A#</td>
<td>B#</td>
<td>C#</td>
</tr>
<tr>
<td>Palm</td>
<td>1.38 ± 0.45</td>
<td>2.49 ± 0.28</td>
<td>2.56 ± 0.36</td>
</tr>
<tr>
<td></td>
<td>(52%)</td>
<td>(100%)</td>
<td>(100%)</td>
</tr>
<tr>
<td>Finger</td>
<td>0.90 ± 0.23</td>
<td>4.13 ± 1.16</td>
<td>4.21 ± 1.23</td>
</tr>
<tr>
<td></td>
<td>(16%)</td>
<td>(77%)</td>
<td>(80%)</td>
</tr>
</tbody>
</table>

Note # A) Hand washing with unmedicated soap, B) Hand washing with antiseptic soap, C) Hand washing with unmedicated soap and alcohol-based hand rub, D) Alcohol-based hand rub.
* Significant at p-value ≤ 0.05, a = One way Analysis of variance, b = Multiple Comparisons (LSD)
to quantitate the number of bacteria when compared to the palm and finger imprints. It is more convenient to perform during routine care activities when compared to the glove juice techniques.40-42

Knowledge of bacteria skin flora is essential to understand the different objectives of hand hygiene approaches. Skin can be the habitat of various microorganisms, mostly bacteria. The species and the amount of bacteria flora found on the skin are different on various parts of the body and may be varied by gender, age, nutritional status, health, and environment. Different skin flora may be found on healthcare workers, since they expose to more various pathogenic microorganisms from patients. In this study, the total bacterial counts on the hands of the dental assistants ranged from $0.4 \times 10^{1}$ to $2.1 \times 10^{2}$ CFU/cm$^2$, with the mean of $4.18 \times 10^{1}$ CFU/cm$^2$. Lucet et al.34 found the bacteria on the hand, ranged from $0.6 \times 10^{2}$ to $0.1 \times 10^{3}$ CFU/hand. Boyce et al.43 reported the higher bacterial counts on the hands of HCWs, ranged from $3.9 \times 10^{4}$ to $4.6 \times 10^{6}$ CFU/cm$^2$. We are aware that the amount of bacteria on the palm is low; this might be due to the fact that the study was designed in real dental setting that dental staff always wear gloves while treating patients. Wearing gloves during working significantly reduce hand contamination.44 Other factors associated with hand contamination were duration and type of care, and methods of hand washing.44 In addition the different amount might also be due to the method of sample collection, i.e., imprints of palm and fingertip, glove juice technique, the method of bacteria culture and enumeration. Moreover our results showed that the amount of the bacteria on the fingertips was much higher than the palms. The total bacterial counts on the fingers of the dental assistants ranged from $1.5 \times 10^{1}$ to $4.8 \times 10^{5}$ CFU/cm$^2$, with the mean of $1.93 \times 10^{5}$ CFU/cm$^2$. The nails seem to be the retention site of bacteria colonization.45 We found that the longer the nails were, the higher the number of bacteria.

The purpose of hand hygiene is to remove the transient flora, particularly pathogens, from the skin in order to preventing cross contamination and cross infection. This study showed all 4 hand hygiene procedures, i.e., washing with unmedicated soap, antiseptic soap, alcohol-based hand rub, and washing with unmedicated soap and ABHR, can significantly reduce the amount of bacteria on the palms and the fingers. However, when comparing the efficacies, we found that hand washing with water and unmedicated soap was the least effective method. Thus, it does not reliably remove pathogenic bacteria from hands. Hand washing with antiseptic soap (chlorhexidine gluconate 4%) and ABHR, did not show any different efficacy. Similar results when compared to hand washing with unmedicated soap and follow with ABHR. According to other studies, alcohol-based hand rub could reduce the number of bacterial more than chlorhexidine gluconate 4% and un-medicated soap30, 32, 34, 44. The use of un-medicated soap with alcohol rub might not be necessary since it increase the cost and take more steps. In this study the dental assistants were least likely to use the un-medicated soap with alcohol-rub due to unpractical steps.

Regarding the guidelines for hand hygiene procedures, a routine method for nonsurgical dental procedures is washing with plain soap or antimicrobial soap and water. However, several reports also showed that hand hygiene compliance increased with the use of ABHR8, 22, 31, 35, 36, 45. Alcohol-based hand rub is waterless antiseptic agents, and acceptable choice if the hands are not visibly soiled. It is rapidly effective against most skin flora easy and fast to use, and do not require water and washing facilities, less irritation. Some studies reported an improved compliance of healthcare workers
using gel formulations\textsuperscript{46}. In some reports the higher compliance and acceptability were explained with better skin care properties and tolerance of the gel over the liquid formulation\textsuperscript{45}. It is thus believed that healthcare workers prefer gel formulations. The studies on compliance of hand hygiene in dental healthcare workers were similar to those in hospital setting in that the levels are still poor\textsuperscript{28, 29, 31}. Although the most popular agent for hand hygiene in a dental hospital was ABHR, only a smaller number of general practice dentists used ABHR frequently\textsuperscript{28}. The study carried out during consecutive use in routine patient care over a period of 1 week, indicated that ABHR was accepted by dental students\textsuperscript{31}. The choice of hand washing using antiseptic soap or ABHR should be made by comparing their pros and cons as well as the cost.

Our results showed the difference between the efficacy of bacteria reduction on the palm and the fingers. For hand hygiene efficacy, disinfectants should be applied to all parts of the hands; particular attention should be made to fingertips and under nails. Therefore, technique is of importance in hand hygiene and training should be provided to the staff\textsuperscript{47}. Huber et al.\textsuperscript{27} suggested the use of alcohol-based hand rub protocol for hand hygiene in dental setting because less costly and less time consuming when compared to traditional hand washing. We found that the cost of using hand washing with medicated soap was comparable to ABHR. We cannot definitely state ABHR is less time consuming because we controlled the washing time of 1 min. In term of duration, it has been reported that time 30 seconds seem to be sufficient for ABHR but may not be long enough for hand washing with a medicated soap\textsuperscript{33, 34}. However, good technique is required to ensure the efficacy of alcohol handrub since some areas are frequently missed. It is also recommended that ABHR should not be used in the presence of visible soil or organic material. For oral surgical procedures which are invasive in nature, the high level of hand hygiene is need, for example an antiseptic soap with residual activity.

In dental settings ventilation and air-conditioning system should be concerned in order to reduce contamination of a dental environment\textsuperscript{23}. Regarding the indoor air quality of this dental clinic, the air ventilation was managed by the central air conditioning systems. In this study, the dental clinic had the temperature and carbon dioxide levels that meet the American Society of Heating, Refrigerating and Air Conditioning Engineers (ASHRAE) suggestion\textsuperscript{48}. However, the relative humidity is little over than the recommended level which might favor growth of microorganisms. The microbiological quality of air in a dental clinic which is affected by dental aerosols and splatters should be routinely monitored in order to assess risks\textsuperscript{23, 37, 38}. To reducing air contamination air filters in air-condition devices should be used. Usually heating, ventilation, and air conditioning (HVAC) systems control temperature and humidity. Thus, air-handling systems should be adequate to control air quality; it must be re-evaluated and upgraded as need.

The limitations of our study should be pointed out. Firstly we did not identified types of contaminated bacteria on hand. There are several studies reported the predominant bacteria on skin were coagulase-negative Staphylococci, \textit{Corynbacterium} spp, \textit{Micrrococcus} spp, \textit{Bacillus} spp.\textsuperscript{32}. On the hands of HCWs, pathogenic flora, i.e., \textit{S. aureus}, gram negative bacilli, enterococci, and yeasts, have been isolated\textsuperscript{5, 49}. Secondly the sample size was small and we did not perform the study in other dental staff, i.e., dentists, dental students. This is due to high patient care in the clinic. Thirdly we did not evaluate the factors influenced the efficacy of hand washing. It has been reported that the
efficacy is influenced by years of experience in infection controls, correct hand washing techniques, time spent on washing. These should be the topics for further studies in dental settings.

In conclusion, our study highlights the efficacy of ABHR gel formulation which is a valid and acceptable hand washing method. It can be used for routine hand washing methods and may lead to increase the compliance of hand hygiene in a dental setting.

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Competing interest: None

Ethical approval: Committee on Human Rights Related to Human Experimentation, Faculty of Dentistry/Faculty of Pharmacy, Mahidol University, Bangkok (COA.No.MU-DT/PY-IRB 2015/003.3001).

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M Dent J Volume 36 Number 1 January-April 2016

12

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12


