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Original

Effectiveness of Handwashing with Electrolyzed Water and Its Comparison with Several Types of Handwashing Methods against Bacteria on Hands after Nursing Procedures

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To examine the antibacterial effectiveness of handwashing with electrolyzed water (EW) when everyday nursing procedures are performed, the cleansing effectiveness of washing for 10s or 30s with EW, washing with 7.5% (w/v) povidone-iodine liquid (PI-washing), washing with medicated liquid soap (mls-washing), and washing with non-antiseptic, plain liquid soap (ls-washing) was compared. When the bacterial counts from hands after nursing procedures were 10^3cfu/hand or greater, it was suggested that washing for 10s or 30s with EW could be expected to have the same level of washing effectiveness as that for mls-washing or ls-washing. Compared to PI-washing, however, the survival ratios were higher. When the bacterial counts from hands were less than 10^3cfu/hand after nursing procedures, washing for 10s or 30s with EW was more effective than ls-washing. Furthermore, EW could keep the bacterial counts from hands as low as those after PI-washing. Based on the above results, washing with EW was as effective as mls-washing, and though slightly less effective than PI-washing, it was considered that the same level of effectiveness seen with PI-washing could be expected for relatively lightly contaminated hands after daily nursing procedures.

Key words : Electrolyzed water/Povidone-iodine/Handwashing/Nursing procedures/Infection control.

INTRODUCTION

As a cause of nosocomial infection transmission, there have been cases in which the hands of hospital personnel served as the medium of contamination. Handwashing by hospital personnel, in particular by nurses, is a procedure that should be performed without fail, and the most effective measurement for the prevention of nosocomial infection. Basically, regular handwashing is done with soap and tap water in the wards. However, handwashing using an antiseptic is a common practice before and after treatment involving close contact with a patient or to promote asepticism. Skin problems due to frequent handwashing with antiseptics, however, are a serious concern for hospital personnel, particularly nurses (Takamori et al., 1992).

Electrolyzed water is ionized water containing hypochlorite produced when electrolysis is conducted by adding an ancillary agent such as salt into tap water. It is known for its strong efficacy against various kinds of peccant bacteria and fungi (Hotta et al.,1994; Middleton et al., 2000 ; Shetty et al., 1999 ; Takeshita and Ando, 2001 ; Zinkevich et al., 2000). Therefore, it has been increasingly expected that such electrolyzed water could be used for handwashing by hospital personnel since it is very safe for the skin

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(Iwasawa and Nakamura, 1995 ; Suzuki et al., 1997). Various reports have been made concerning the effectiveness of handwashing with electrolyzed water (Hitomi et al., 1998 ; Kasuda et al., 1997 ; Takeshita et al., 2001 ; Yamamoto et al., 2000). However, very few of them have reported the cleansing effectiveness against bacteria on hands at actual nursing sites (Takeshita et al., 2001).

In this study, then, in order to examine the handwashing effectiveness in cases where electrolyzed water was used for handwashing after nursing procedures, quantitative comparisons were made in the cleansing effectiveness of various washing agents including the kinds of soap commonly used in wards and 7.5% (w/v) povidone-iodine liquid.

MATERIALS AND METHODS

Preparation of electrolyzed water

Electrolyzed water was produced by using "ACID WATER PRODUCTION APPARATUS®" (TOTO Ltd., TFS400A model, non-diaphragm, running-water type). Previous reports by the authors were used for electrolysis conditions (Takeshita et al., 2001). A pH meter (Horiba, Ltd., F-14) was used for measuring pH of the produced electrolyzed water, and a residual chlorine meter (HACH Co., 46700-00) was used to measure free residual chlorine, respectively. The water quality of the electrolyzed water was pH6.0-6.5, and the free residual chlorine concentration was 18.0-20.0mg/L.

Nursing subject

A long-term hospitalized patient with no nosocomial infection who was hospitalized in the physician's ward of hospital A was chosen as the nursing procedure subject. Two nurses conducted various daily nursing procedures performed in the ward including endotracheal aspiration, bed baths, changing positions, diapering, oral care, and genital washing. Sterile gloves were worn for the endotracheal aspiration due to the requirement for an aseptic procedure. In addition, when heavy hand contamination was expected from the patient's secretions or excretions during nursing procedures such as genital washing, the nurses wore a pair of gloves before the nursing procedure.

Method for counting the bacteria on the hands

First, a sampling of bacteria on the hands after performing a nursing procedure was conducted. Two ml of physiological saline was used for the sampling of bacteria, and the bacteria on the hands were collected from both the right and left palms of the nurses by using sterilized swabs. As for procedures performed with gloves, bacteria on the hands were collected from both the right and left palms after the nurses removed the gloves when the nursing procedure was finished. Handwashing was performed by rubbing hands together well under running electrolyzed water (3.0L/min) produced by "ACID WATER PRODUCTION APPARATUS®" right after the sampling. A paper towel was used to dry off the hands, and the bacteria remaining on the right and left palms were collected by sterile swabs with 2 ml of physiological saline. The collected samples from both palms of the nurses after the nursing procedures and handwashing were diluted appropriately, and smeared into ovine blood agar (Nissui Plate Sheep Blood Agar®; Nissui Pharmaceutical, Co., Ltd.). After 48-h aerobic incubation at 36℃, bacterial counts were calculated by counting the bacterial numbers. The handwashing time was set at 10s and 30s.

Methods of washing with electrolyzed water, liquid soap, medicated liquid soap and 7.5% (w/v) povidone-iodine liquid

The washing methods used were general handwashing methods used in wards, and the following handwashing materials were used: commercial liquid soap with no added sterilizing ingredient (1 ml) and tap water (3.0L/min) (hereinafter referred to as ls-washing); commercial liquid soap containing triclocarban and triclosan as sterilizing ingredients and tap water (3.0L/min) (mls-washing); and 7.5% (w/v) povidone-iodine liquid and tap water (3.0L/min) (PI-washing). Furthermore, after the PI-washing, 2 ml of 0.1% sodium thiosulfate solution instead of physiological saline was used for the collection. Nurses performed fifteen seconds of rubbing and washing with ls-washing, mls-washing and PI-washing, followed by a 15s rinsing with tap water. Furthermore, washing for 10s with EW (sampling counts=36), washing for 30s with EW (sampling counts=36), 30-s ls-washing (sampling counts=36), 30-s-mls-washing (sampling counts=28), and 30-s-PI-washing (sampling counts=36) were conducted alternately every day, and the same handwashing method was used for the same day.

Index for washing effectiveness

As an index for the washing effectiveness of handwashing, the following survival ratio was defined as the logarithmic decrement calculated from detected bacterial counts after handwashing (N) and bacterial counts from hands after a nursing procedure (N_0).

$$\text{Survival ratio} = \log_{10} (N/N_0)$$

Furthermore, Scheffe's multiple comparison test was used as a statistical test.

RESULTS

In this study, the mean±standard deviation of the bacterial counts from hands after nursing procedures for all the samples was 3.14±0.85 (log cfu/hand). First, the bacterial counts from hands after the nursing procedures were divided into those greater and those less than 10^3 cfu/hand, and the washing effectiveness was evaluated for cases of higher and lower hand bacterial counts after the nursing procedures.

Washing effectiveness in the cases where the bacterial counts from hands after nursing procedures were 10^3 cfu/hand or greater

Table 1 shows the mean±standard deviation with regard to the bacterial counts from hands after the nursing procedures, and the detected bacterial counts from hands and survival ratios after handwashing with each washing method. Table 2 shows the result of Scheffe's multiple comparison tests concerning the bacterial counts from hands after the nursing proce-

TABLE 1. Cleansing effectiveness of handwashing with various methods in the cases where the bacterial counts from hands after nursing procedures were 10^3cfu/hand or greater.

Washing method	No. of samples	Bacterial counts from hands (log cfu/hand)		Survival ratio
		After nursing procedures	After handwashing	
Ls-washing	19	3.69±0.35[a]	2.51±0.59	−1.18±0.63
Mls-washing	10	3.52±0.38	2.24±0.34	−1.28±0.38
Washing for 30s with EW	14	3.52±0.47	1.86±0.59	−1.66±0.76
Washing for 10s with EW	21	3.93±0.61	2.30±0.75	−1.63±0.61
Pl-washing	21	4.16±0.82	1.68±0.71	−2.48±0.90

[a]Mean±S.D.

TABLE 2. P-value of Scheffe's multiple comparison test in the cases where the bacterial counts from hands after nursing procedures were 10^3cfu/hand or greater.

Washing method	Washing method				
	Ls-washing	Mls-washing	Washing for 30s with EW	Washing for 10s with EW	Pl-washing
Ls-washing					
Mls-washing	0.9669 0.8876 0.9980				
Washing for 30s with EW	0.9567 0.0950 0.4337	1.0000 0.7269 0.7775			
Washing for 10s with EW	0.7907 0.8977 0.3961	0.5032 0.9996 0.7870	0.4029 0.4263 0.9999		
Pl-washing	0.1721 0.0046[b] <0.0001[c]	0.0936 0.2919 0.0013[b]	0.0476[a] 0.9607 0.0307[a]	0.7957 0.0587 0.0069[b]	

Each line indicates the *P* value concerning the bacterial counts from hands after nursing procedures, after handwashing, and the survival ratio, respectively.
[a]Statistically significant at 5%.
[b]Statistically significant at 1%.
[c]Statistically significant at 0.1%.

dures, and the detected bacterial counts and survival ratios after handwashing.

According to Tables 1 and 2, the bacterial counts from hands (log cfu/hand) after the nursing procedures, on those days when ls-washing, mls-washing, washing for 30s with EW, washing for 10s with EW, and Pl-washing were performed were 3.69 ± 0.35, 3.52±0.38, 3.52±0.47, 3.93±0.61, and 4.16±0.82, respectively. A significant difference depending on the day was found. On the day when washing for 30s with EW was conducted, the bacterial counts from hands were less than on the day when Pl-washing was conducted ($p<0.05$). When ls-washing, mls-washing, washing for 30s with EW, washing for 10s with EW, and Pl-washing were performed, the detected bacterial counts (log cfu/hand) following each washing method were 2.51±0.59, 2.24±0.34, 1.86 ±0.59, 2.30±0.75, and 1.68±0.71, respectively, demonstrating a significant difference. As for the detected bacterial counts after washing for 30s with EW and washing for 10s with EW, no significant difference was found compared to ls-washing, mls-washing, and Pl-washing. Higher detected bacterial counts were found after ls-washing ($p<0.01$) compared to the Pl-washing.

Survival ratios were compared after handwashing according to each handwashing method. When ls-washing, mls-washing, washing for 30s with EW, washing for 10s with EW and Pl-washing were conducted, the survival ratios were −1.18±0.63, −1.28 ±0.38, −1.66±0.76, −1.63±0.61, and −2.48± 0.90, respectively, demonstrating a significant difference depending on the handwashing method. The survival ratios for washing for 30s with EW ($p<0.05$) and washing for 10s with EW ($p<0.01$) were higher than for Pl-washing. The survival ratio after Pl-washing was lower than those after ls-washing ($p<0.001$) and mls-washing ($p<0.01$), showing the lowest survival ratio among all of the washing methods.

Washing effectiveness in cases where the bacterial counts from hands after the nursing procedures were less than 10^3cfu/hand

Table 3 shows the mean±standard deviation with regard to the bacterial counts from hands after the nursing procedures, and the detected hand bacterial counts and survival ratios after handwashing using each handwashing method. Table 4 shows Scheffe's multiple comparison test results concerning the bacterial counts from hands after the nursing procedures, and the detected hand bacterial counts and survival ratios after handwashing.

According to Tables 3 and 4, the bacterial counts from hands (log cfu/hand) after the nursing procedures on those days when ls-washing, mls-washing, washing for 30s with EW, washing for 10s with EW, and Pl-washing were performed were 2.47 ± 0.36, 2.23±0.60, 2.56±0.23, 2.63±0.33, and 2.52±0.28, respectively. No significant difference depending on the day was found. The detected bacterial counts (log cfu/hand) after handwashing with each method on the days when ls-washing, mls-washing, washing for 30s with EW, washing for 10s with EW, and Pl washing were performed were 2.28±0.59, 1.49± 0.48, 1.56±0.47, 1.48±0.40, and 1.23±0.44, respectively. A significant difference depending on the handwashing method was found. After washing for 30s with EW ($p<0.001$) and washing for 10s with EW ($p<0.001$), the detected hand bacterial counts were less compared to those after ls-washing, but no significant difference was found between mls-washing and Pl-washing. The detected bacterial counts after ls-washing were higher than after using the other

TABLE 3. Cleansing effectiveness of handwashing with various methods in the cases where the bacterial counts from hands after nursing procedures were less than 10^3cfu/hand.

Washing method	No. of samples	Bacterial counts from hands (log cfu/hand)		Survival ratio
		After nursing procedures	After handwashing	
Ls-washing	17	2.47±0.36[a]	2.28±0.59	−0.19±0.69
Mls-washing	18	2.23±0.60	1.49±0.48	−0.74±0.83
Washing for 30s with EW	22	2.56±0.23	1.56±0.47	−0.99±0.59
Washing for 10s with EW	15	2.63±0.33	1.48±0.40	−1.15±0.38
Pl-washing	15	2.52±0.28	1.23±0.44	−1.29±0.46

[a]Mean±S.D.

TABLE 4. P-value of Scheffe's multiple comparison test in the cases where the bacterial counts from hands after nursing procedures were less than 10^3cfu/hand.

Washing method	Washing method				
	Ls-washing	Mls-washing	Washing for 30s with EW	Washing for 10s with EW	Pl-washing
Ls-washing					
Mls-washing	0.4621 0.0003[b] 0.1322				
Washing for 30s with EW	0.9788 0.0007[b] 0.0036[a]	0.1314 0.9929 0.7877			
Washing for 10s with EW	0.8588 0.0005[b] 0.0011[a]	0.0698 0.9999 0.4438	0.9878 0.9907 0.9608		
Pl-washing	0.9980 <0.0001[b] 0.0001[b]	0.3084 0.6838 0.1610	0.9994 0.3859 0.7070	0.9647 0.7469 0.9826	

Each line indicates the P value concerning the bacterial counts from hands after nursing procedures, after handwashing, and the survival ratio, respectively.
[a]Statistically significant at 1%.
[b]Statistically significant at 0.1%.

washing methods ($p<0.001$).

The survival ratios after handwashing with each method were compared. When ls-washing, mls-washing, washing for 30s with EW, washing for 10s with EW and Pl-washing were conducted, the survival ratios were -0.19 ± 0.69, -0.74 ± 0.83, -0.99 ± 0.59, -1.15 ± 0.38, and -1.29 ± 0.46, respectively, demonstrating a significant difference depending on the handwashing method. The survival ratios after both washing for 30s with EW ($p<0.01$) and washing for 10s with EW ($p<0.01$) were lower compared to those after ls-washing, but no significant difference was found between those after mls-washing and Pl-washing.

Analysis of all the data

Table 5 shows the mean±standard deviation for all the data with regard to the bacterial counts from hands after the nursing procedures, and the counts of detected bacteria and survival ratios after handwashing using each handwashing method. Table 6 shows Scheffe's multiple comparison test results concerning the bacterial counts from hands after the nursing procedures, and the detected bacterial

TABLE 5. Cleansing effectiveness of handwashing with various methods for all the data with regard to the bacterial counts from hands after nursing procedures.

Washing method	No. of samples	Bacterial counts from hands (log cfu/hand)		Survival ratio
		After nursing procedures	After handwashing	
Ls-washing	36	3.11±0.71[a]	2.40±0.59	−0.71±0.79
Mls-washing	28	2.69±0.67	1.76±0.57	−0.93±0.74
Washing for 30s with EW	36	2.93±0.59	1.68±0.53	−1.25±0.73
Washing for 10s with EW	36	3.39±0.82	1.96±0.74	−1.43±0.57
Pl-washing	36	3.48±1.04	1.50±0.65	−1.98±0.94

[a]Mean±S.D.

TABLE 6. P-value of Scheffe's multiple comparison test for all the data with regard to the bacterial counts from hands after nursing procedures.

Washing method	Washing method				
	Ls-washing	Mls-washing	Washing for 30s with EW	Washing for 10s with EW	Pl-washing
Ls-washing					
Mls-washing	0.3618 0.0026[b] 0.8596				
Washing for 30s with EW	0.9208 0.0001[c] 0.0660	0.8401 0.9929 0.5996			
Washing for 10s with EW	0.7296 0.0588 0.0043[b]	0.0224[a] 0.8042 0.1607	0.2281 0.4675 0.9149		
Pl-washing	0.4615 <0.0001[c] <0.0001[c]	0.0060[b] 0.6040 <0.0001[c]	0.0894 0.8209 0.0036[b]	0.9939 0.0481[a] 0.0589	

Each line indicates the P value concerning the bacterial counts from hands after nursing procedures, after handwashing, and the survival ratio, respectively.
[a]Statistically significant at 5%.
[b]Statistically significant at 1%.
[c]Statistically significant at 0.1%.

counts and survival ratios after handwashing. According to Tables 5 and 6, the bacterial counts from hands (log cfu/hand) after the nursing procedures on those days when ls-washing, mls-washing, washing for 30s with EW, washing for 10s with EW, and Pl washing were conducted were 3.11 ± 0.71, 2.69 ± 0.67, 2.93 ± 0.59, 3.39 ± 0.82 and 3.48 ± 1.04, respectively. A significant difference depending on the day was found. When mls-washing was conducted, the bacterial counts from hands were less than those where washing for 10s with EW ($p<0.05$) or Pl-washing ($p<0.01$) was conducted. The bacterial counts (log cfu / hand) detected after handwashing by ls-washing, mls-washing, washing for 30s with EW, washing for 10s with EW, and Pl-washing were 2.40 ± 0.59, 1.76 ± 0.57, 1.68 ± 0.53, 1.96 ± 0.74 and 1.50 ± 0.65, respectively, demonstrating a significant difference according to the washing method. After washing for 10s with EW, the detected hand bacterial counts were greater than those after Pl-washing ($p<0.05$). After washing for 30s with EW, the detected hand bacterial counts were less than those after ls-washing ($p<0.001$). After ls-washing, the detected hand bacterial counts were higher than those after mls-washing ($p<0.01$) and Pl-washing ($p<0.001$).

We compared the survival ratios after handwashing according to the various methods. When ls-washing, mls-washing, washing for 30s with EW, washing for 10s with EW, and Pl-washing were conducted, the survival ratios were -0.71 ± 0.79, -0.93 ± 0.74, -1.25 ± 0.73, -1.43 ± 0.57, and -1.98 ± 0.94, respectively, demonstrating a significant difference depending on the handwashing method. Washing for 10s with EW showed lower survival ratios compared to those for ls-washing ($p<0.01$), while the survival ratios for mls-washing, washing for 30s with EW and Pl-washing did not show a significant difference statistically. Washing for 30s with EW showed higher survival ratios compared to those shown for Pl-washing ($p<0.01$). Pl-washing showed lower survival ratios compared to those shown for ls-washing ($p<0.001$) and mls-washing ($p<0.001$).

DISCUSSION

For the purpose of suggesting the most appropriate handwashing method at a nursing site, there is a method that examines the cleansing effectiveness using various kinds of soap and hand antiseptics on hands which have been artificially contaminated (light contamination: about 10^3cfu/fingertips, heavy contamination : about 10^6cfu/fingertips) presented by Cardoso et al. (1999) and Guihermetti et al. (2001). Regarding the experiment in which *Acinetobacter baumannii*, a nosocomial infectious bacteria, was

artificially applied to hands, it has been reported that when the hand contamination was heavy, washing with a scrub containing 10% (w/v) povidone-iodine was more effective than ls-washing, but when it was light, there was no difference in cleansing effectiveness between the two methods (Cardoso et al., 1999). According to an experiment in which methicillin-resistant *Staphylococcus aureus* (MRSA) was used as an index, it has been reported that washing with a scrub containing 10% (w/v) povidone-iodine showed higher cleansing effectiveness compared to ls-washing in case of both heavy and light contamination (Guihermetti et al., 2001). In this study, the hand bacterial contamination was relatively light on any day conducted, and no heavy hand contamination was found in which the bacterial counts from hands were 10^6cfu/hand or greater. However, there was a question whether there would be a difference in the cleansing effectiveness according to the five washing methods depending on the difference in the bacterial counts from hands after nursing procedures. Thus, an analysis was made by categorizing the bacterial counts from hands after nursing procedures as those indicating relatively heavy contamination (10^3cfu/hand or greater) and light contamination (less than 10^3cfu/hand).

When the bacterial counts from hands after the nursing procedures were 10^3cfu/hand or greater, washing for 10s or 30s with EW showed the same survival ratios as ls-washing and mls-washing. Based on this result, it would be suggested that the same level of cleansing effectiveness from mls-washing and ls-washing can be expected from washing with EW when the bacterial counts on the hands are relatively great. However, it would be also suggested that there would be cases in which the bacteria on the hands could not be completely removed in a similar manner as with mls-washing or ls-washing. In the meantime, it was suggested that Pl-washing could reliably remove bacteria from hands even when the initial bacterial counts were great. Alyliffe et al. (1988) have reported that detergent containing antiseptic is more effective than liquid soap, and that the most effective one is that containing chlorhexidine or povidone-iodine, supporting the results of this study. However, there was a tendency in which the bacterial counts from hands detected after washing for 30s with EW were as low as those after Pl-washing. Based on this result, the possibility is suggested that washing with EW would maintain low levels of bacterial flora on the hands, even when the bacterial counts on the hands are relatively great.

When the bacterial counts from hands after nursing procedures were less than 10^3 cfu/hand, washing for 10s or 30s with EW showed survival ratios as low as those after mls-washing and Pl-washing. Based on this result, it could be considered that washing for 10s or 30s with EW could keep the bacterial counts on hands after handwashing as low as in the case of Pl-washing when the bacterial counts on the hands after the nursing procedure were relatively low, and that the same level of cleansing effectiveness could be expected as that of Pl-washing, as well as of mls-washing. Cardoso et al., (1999) reported that when hand contamination was heavy, washing with a scrub containing 10% povidone-iodine was more effective than liquid soap, but when the contamination was light, there was no difference in cleansing effectiveness between them. In the results of this study, the survival ratios after ls-washing were higher than after any other hand-washing method. As a reason for this, it was considered that there were cases in which resident bacterial flora on hands were detected in the collection method used in the present study. Furthermore, it could be that there were relatively many cases in which bacteria had risen to the surface of the hands after ls-washing. In this study, no *Staphylococcus aureus* or MRSA causing nosocomial infections, were detected before the procedures from the hands of the two nurses who performed the nursing. If, however, those bacteria were found, there might be cases in which these bacteria may have risen after ls-washing. It was therefore considered that sufficient care was required. In the meantime, compared to ls-washing or mls-washing, washing for 10s with EW did not show any cases where the detected bacteria increased after handwashing: it was thus considered that a short period of washing could maintain low bacterial counts on hands.

Finally, comparisons were made with all the data. Washing for 10s with EW was more effective in cleansing than ls-washing, and it could be expected to have the same level of washing effectiveness as mls-washing, washing for 30s with EW, and Pl-washing. However, the bacterial counts detected after washing for 10s with EW were higher than after Pl-washing. Based on these results, it was considered that washing with EW has slightly inferior effectiveness compared to Pl-washing. In the meantime, approximately 83% of all of the samples of the hand bacterial counts after nursing procedures were less than 10^4cfu/hand. It appears that direct hand contamination was relatively light because gloves were worn before performing the nursing procedures when heavy hand contamination would be expected with the patient's secretions or excretions. When the bacterial counts from hands after nursing procedures were in the range of less than 10^4 cfu/hand, no significant

statistical differences were found in terms of the survival ratios and the detected bacterial counts between handwashing for 30s or 10s with EW and PI-washing. Based on these, it was considered that the same level of effectiveness could be expected with washing with EW as with PI-washing for hands when there is relatively light contamination of up to 10^4cfu/hand or so after routine nursing procedures.

From the above mentioned results, washing with EW seems to be as effective as mls-washing, and though it seems slightly less effective compared to PI-washing, it was considered that the same level of effectiveness as PI-washing could be expected for relatively lightly contaminated hands after daily nursing procedures.

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