

# Evaluation of Two Different Hand Hygiene Procedures during Routine Patient Care

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In this study, the antimicrobial efficacy of hand washing (HW) and hand washing plus rubbing with an alcohol-based solution (HWR) on numbers of total and transient flora colonies on the hands of healthcare workers (HCWs) during routine patient care was assessed. Samples were collected, using a standard bag broth technique, from the hands of 154 HCWs, before and immediately after carrying out a hand hygiene procedure. The numbers of total and transient flora colonies per plate were counted and transient pathogens were identified. A significant

statistical difference between ward speciality was detected with respect to the isolation rate of transient flora. Transient hand flora were recovered from 25.3% of HCWs before carrying out the hand hygiene procedure. With respect to the disappearance and prevention of regrowth of transient flora after hand hygiene, the HWR technique was significantly more effective than HW. In conclusion, a disinfectant should be added to the hand washing process to achieve optimum protection against nosocomial infections in routine hospital practice.

**KEY WORDS:** INFECTION CONTROL; HAND WASHING; HAND RUBBING; ALCOHOL-BASED RUB; HAND FLORA

## Introduction

The role of contamination via the hands of healthcare workers (HCWs) in the transmission of infection to patients has long been recognized. Hand washing has been shown to decrease the transmission of organisms between patients and HCWs.<sup>1</sup> Compliance with hand washing using soap and water by HCWs has, however, been measured as < 50% in most observational studies in European and American hospitals.<sup>2</sup>

Hand hygiene is considered to be the most important tool in nosocomial infection

control.<sup>3,4</sup> There are three methods for the post-contamination treatment of hands: (i) social hand wash, which is the cleaning of hands with plain, non-medicated bar or liquid soap and water for removal of dirt, soil and various organic substances; (ii) hygienic or antiseptic hand wash, which is the cleaning of hands with antimicrobial soaps containing a single active agent, which are usually available as liquid preparations; and (iii) hygienic hand disinfection, which normally consists of the application of an alcohol-based hand rub onto dry hands without the use of water.<sup>5</sup>

The introduction of alcohol-based hand rubs has been shown significantly to improve compliance to hand hygiene among HCWs in some hospitals and to decrease overall nosocomial infection rates.<sup>6,7</sup> From laboratory investigations, the antimicrobial effect of alcohol-based hand-hygiene products has been documented to be superior to hand washing.<sup>8,9</sup> This prompted the present study to assess the comparative microbiological efficiency of hand washing (HW) and hand washing and rubbing with an alcohol-based solution (HWR) by HCWs in intensive care units (ICUs) and non-ICU areas, with specific emphasis on transient flora. The study also aimed to determine the predisposing factors for hand contamination after patient care in daily hospital practice.

## Subjects and methods

### STUDY DESIGN

This prospective study was conducted at the Microbiology Department of Gaziantep University Hospital (Gaziantep, Turkey), which is an 800-bed teaching hospital, during a 2-month period from January to March 2008. It was approved by the Medical Ethics Committee of Gaziantep University (report number 10-2007/62).

### CLASSIFICATION OF HCWs

The following information was recorded for each HCW: gender, job title (nurse, physician, medical student, hospital attendant/nursing assistant, cleaning personnel), type of ward they worked on, ward speciality and the type of healthcare procedure performed before hand hygiene was implemented. All HCWs enrolled into the study gave written consent to participate.

### HAND HYGIENE PROCEDURES

The HCWs were divided into two groups: HW was practiced in one group and HWR was

practiced in the other group. All HCWs were given information about both hand hygiene techniques. The HW procedure was considered to be satisfactory if both hands were washed together with unmedicated soap for  $30 \pm 5$  s, rinsed under running water and then dried with a paper towel.<sup>10</sup> The HWR technique was performed by initial hand washing with unmedicated soap and water, drying with a paper towel, followed by disinfection with an alcohol-based solution (Biodex; Tarko-Hijyenmarket, Istanbul, Turkey), containing 77% ethyl alcohol, benzalkonium chloride, glycerine, fragrance and water, until dry (around 30 s) without using a paper towel to accelerate drying. Hand-rub dispensers and unmedicated soap dispensers were located in every patient's room. All sinks in the hospital were equipped with unmedicated soap and sinks were located in nursing stations.

### MICROBIOLOGICAL SAMPLING AND PROCESSING

The hands of the HCWs were sampled simultaneously before and immediately after the hand hygiene procedure was performed. A standard bag broth technique was employed.<sup>11,12</sup> Each hand was sequentially immersed in 50 ml of brain-heart infusion broth in a sterile plastic bag and kneaded for 30 s. After removal of the hands from the bag, the broth was transferred into a sterile container. For the samples taken after hand hygiene, 3% Tween and 0.3% lecithin were added to the sampling solution in order to neutralize residual antiseptics on the hands.<sup>13</sup>

Each sample was inoculated onto agar either within 1 h or after storage at 4 °C for up to 5 h. An inoculum of 0.1 ml of sample (undiluted, 1:10 and 1:100 dilutions) was plated onto the following agar media: Columbia sheep blood agar (5%), eosin

methylene blue agar, Sabouraud dextrose agar with chloramphenicol and gentamicin, and bile esculin agar. Plates were incubated for up to 48 h for bacterial growth and for up to 7 days for yeast growth at 37 °C under aerobic conditions. Results were expressed as the number of colony-forming units (CFUs). Samples with no visible growth were considered negative. All colony counts were transformed into decimal logarithms.

For the identification of micro-organisms grown on culture media, both automated methods (VITEK® 2 and API ID 32C®; bioMérieux, Marcy l'Etoile, France) and conventional methods were used. Micro-organisms other than coagulase-negative staphylococci, *Corynebacterium* spp, *Micrococcus* spp and *Bacillus* spp were considered to be elements of transient flora and, therefore, potentially pathogenic.

### STATISTICAL ANALYSES

Statistical analyses were carried out using the SPSS® statistical package, version 13.0 (SPSS Inc., Chicago, IL, USA) for Windows®. Colony counts from hands were compared using logarithm ( $\log_e$ )-transformed microbiological counts because of log-normal distribution of the variable. When no colonies were detected, values were set to 0.5 (half of the minimum value) in order to apply logarithm transformation. Categorical data were presented as number and frequency, and continuous data as mean  $\pm$  SD. Categorical variables were compared by the  $\chi^2$ -test. The independent *t*-test was used to test the difference for total flora colonies between the two genders and between ICU and non-ICU sites before hand hygiene. The Kruskal–Wallis test was used to make comparisons for ward speciality and job titles. The Mann–Whitney *U*-test was performed with respect to growth of transient flora between genders before hand hygiene.

The paired samples *t*-test and Wilcoxon's signed rank test were employed to examine differences in total flora and transient flora between HW and HWR groups before and after hand hygiene. All tests were considered significant at a *P*-value < 0.05.

## Results

### HCWs AND THEIR CLASSIFICATION

Samples were collected from the hands of 154 HCWs (79 females and 75 males) during the 2-month period; 71 were randomized to HW and 83 to HWR. The HCWs were classified according to their job title as nurse ( $n = 55$ ), physician ( $n = 33$ ), medical student ( $n = 11$ ), hospital attendant/nursing assistant ( $n = 25$ ) and cleaning personnel ( $n = 30$ ). They were further classified according to their wards as follows: surgical ( $n = 48$ ); coronary unit and internal medicine ICU ( $n = 32$ ); internal medicine and cardiology ( $n = 23$ ); paediatrics and newborn ( $n = 21$ ); adult oncology ( $n = 11$ ); surgical ICU ( $n = 10$ ); and paediatric oncology ( $n = 9$ ). Forty-two (27.3%) of the HCWs were working in ICUs and the others worked in non-ICU medical wards.

### TOTAL FLORA

Factors associated with hand contamination (total flora) before HW or HWR are given in Table 1. When the total flora of HCWs was assessed with respect to mean colony count, significant differences between males and females ( $P = 0.002$ ), job titles ( $P = 0.012$ ), type of ward ( $P = 0.001$ ) and ward speciality ( $P = 0.031$ ) were observed. The hands of female HCWs were found to be less contaminated than the hands of male HCWs. The hands of nurses were found to be less contaminated than those of nursing assistants. The mean total colony count detected on the hands of HCWs in paediatric and newborn wards was found to be less than that of HCWs working

**TABLE 1:**  
Factors affecting hand contamination (total number of hand flora colonies) among healthcare workers (HCWs) before the use of hand hygiene procedures

Variables	No. of HCWs	Total No. of hand flora colonies (CFU/ml)	Statistical significance
Gender			$P = 0.002^a$
Male	75	$6.63 \pm 1.31$	
Female	79	$5.74 \pm 2.11$	
Job title			$P = 0.012^b$
Nurse	55	$5.51 \pm 2.36$	
Physician	33	$6.19 \pm 1.28$	
Cleaning personnel	30	$6.50 \pm 1.70$	
Hospital attendant/nursing assistant	25	$6.95 \pm 0.88$	
Medical student	11	$6.78 \pm 0.56$	
Type of ward			$P = 0.001^a$
Intensive care unit (ICU)	42	$6.76 \pm 0.95$	
Medical	112	$5.95 \pm 2.01$	
Ward speciality			$P = 0.031^b$
Paediatrics and newborn	21	$5.37 \pm 1.54$	
Internal medicine and cardiology	23	$6.36 \pm 1.21$	
Coronary unit and internal medicine – ICU	32	$6.63 \pm 0.96$	
Surgical ICU	10	$7.19 \pm 0.82$	
Surgical	48	$5.85 \pm 2.46$	
Adult oncology	11	$6.91 \pm 1.08$	
Paediatric oncology	9	$5.60 \pm 2.49$	

Data are presented as mean  $\pm$  SD.<sup>a</sup>Independent *t*-test; <sup>b</sup>Kruskal–Wallis test.

in other wards. When the wards were assessed on the basis of ICU and non-ICU, the mean number of total flora colonies growing on the hands of HCWs working in ICUs was found to be significantly higher than for those working in non-ICUs ( $P = 0.001$ ). The HCWs reported their glove wearing habits during the healthcare procedure preceding the hand washing as: 140 (90.9%) wore gloves, eight (5.2%) did not use gloves and six (3.9%) sometimes used gloves. As there were large differences, these data were not statistically evaluated.

The mean  $\pm$  SD number of total flora colonies recovered from the hands of HCWs was  $6.23 \pm 1.83$  CFU/ml before HW and  $6.11 \pm 1.81$  CFU/ml before HWR. After the

application of HW and HWR, total flora colony numbers decreased to  $4.86 \pm 2.42$  CFU/ml and  $4.43 \pm 2.78$  CFU/ml, respectively. There was a statistically significant difference in the mean number of total flora colonies before and after hand hygiene procedures for both techniques ( $P < 0.001$  for both comparisons); however there was no significant difference between the two techniques. The mean  $\pm$  SD reduction in total flora colonies was significantly greater in female HCWs than male HCWs (males  $1.05 \pm 1.68$  CFU/ml; females  $2.00 \pm 2.64$  CFU/ml;  $P = 0.008$ ), indicating that female HCWs followed the hand hygiene procedure better than males. No statistically significant differences were found between ward

speciality, job title or type of ward with respect to reduction in the number of total flora colonies.

### TRANSIENT FLORA

Single or multiple microorganisms were isolated as transient flora from the hands of 39 (25.3%) of the HCWs before carrying out hand hygiene procedures (Table 2); 14 (19.7%) HCWs were positive for transient flora in the HW group compared with 25 (30.1%) in the HWR group. No statistically significant difference was observed between gender, job title or type of ward with respect to isolation rates of transient flora. A significant difference was, however, observed with respect to ward speciality ( $P = 0.026$ ). The difference was particularly marked in

the paediatric oncology ward, in which the prevalence of transient flora on the hands of HCWs was found to be higher than those working in other wards.

The transient flora on the hands of the 39 HCWs in the HW and HWR groups, respectively, were: *Candida* spp ( $n = 4$ ,  $n = 7$ ); *Acinetobacter* spp ( $n = 3$ ,  $n = 6$ ); *Enterococcus* spp ( $n = 4$ ,  $n = 5$ ); *Staphylococcus aureus* ( $n = 1$ ,  $n = 4$ ); *Pseudomonas aeruginosa* ( $n = 1$ ,  $n = 3$ ); *Enterobacter* spp ( $n = 3$ ,  $n = 1$ ); *Serratia marcescens* ( $n = 1$ ,  $n = 2$ ); and *Streptococcus pneumoniae* ( $n = 0$ ,  $n = 2$ ). Two of the five *S. aureus* strains were found to be methicillin-resistant.

There was a statistically significant difference in the transient flora colony numbers between the HW and HWR groups

**TABLE 2:**  
Factors affecting the incidence transient hand flora among healthcare workers (HCWs) before the use of hand hygiene procedures

Variables	No. of HCWs	No. (%) of HCW with transient flora	Statistical significance
Gender			NS <sup>a</sup>
Male	75	21 (28.0)	
Female	79	18 (22.8)	
Job title			NS <sup>a</sup>
Nurse	55	13 (23.6)	
Physician	33	11 (33.3)	
Cleaning personnel	30	6 (20.0)	
Hospital attendant/nursing assistant	25	7 (28.0)	
Medical students	11	2 (18.2)	
Type of ward			NS <sup>a</sup>
Intensive care unit (ICU)	42	11 (26.2)	
Medical	112	28 (25.0)	
Ward speciality			$P = 0.026^a$
Paediatrics and newborn	21	2 (9.5)	
Internal medicine and cardiology	23	4 (17.4)	
Coronary unit and internal medicine – ICU	32	8 (25.0)	
Surgical ICU	10	3 (30.0)	
Surgical	48	11 (22.9)	
Adult oncology	11	5 (45.5)	
Paediatric oncology	9	6 (66.7)	

NS, not statistical significant ( $P < 0.05$ ).

<sup>a</sup> $\chi^2$ -test.

before and after hand hygiene:  $5.17 \pm 1.59$  CFU/ml before HW and  $1.82 \pm 2.91$  CFU/ml after HW,  $P = 0.001$ ;  $4.34 \pm 1.35$  CFU/ml before HWR and  $0.22 \pm 1.67$  CFU/ml after HWR,  $P < 0.001$ . The number of transient flora colonies after HWR was significantly lower than after HW ( $P = 0.008$ ).

Following hand hygiene, regrowth occurred in seven (50%) of the 14 individuals in the HW group: one each of *S. marcescens*, *S. aureus*, *Acinetobacter* spp, *Enterococcus* spp, *Pseudomonas* spp, *Enterobacter aerogenes* and yeast. Regrowth occurred in four (16%) of the 25 participants who carried out HWR: yeast in one and *S. aureus* in three. When the two groups were compared, the HWR technique was significantly more effective than HW at preventing regrowth ( $P = 0.02$ ). All of the 12 Gram-negative bacilli were cleared following the HWR procedure, whereas only four (50%) of eight were cleared following the HW procedure ( $P = 0.006$ ). No statistically significant difference in efficacy was noted for yeast (six of seven [HW] versus three of four [HWR], respectively).

## Discussion

All patient care activities that involve contact with patients or their body fluids are at risk of microbial contamination. For more than a century, hand hygiene has been accepted as one of the primary ways to control the spread of infectious agents.<sup>14</sup> Prevention of bacterial contamination by transient flora and possible subsequent infection requires timely hand cleansing that may be achieved by washing or disinfecting the hands.<sup>15</sup>

Pittet *et al.*<sup>15</sup> reported that bacterial contamination of HCWs' hands was highest on rehabilitation wards but lowest on orthopaedic surgery wards. Consistent with this finding, reports of nosocomial infections resulting from cross-transmission showed

high infection rates and poor compliance with hand-cleansing practices in rehabilitation wards and long-term care facilities.<sup>16,17</sup> The mean number of total flora colonies in the ICU was significantly more than in non-ICU wards in the present study. Meanwhile, more transient flora were isolated from the hands of HCWs working in the paediatric oncology ward than from the hands of HCWs working in other clinics. We suggest that, since the patients in oncology wards are generally immunosuppressed, more microorganisms can colonize their skin and, therefore, spread to the hands of HCWs during patient care.

Widmer and Dangel<sup>18</sup> evaluated the reduction in the mean number of colonies following use of an alcohol-based handrub in terms of demographic data such as age, gender, job description and job experience, but they did not detect a significant difference between any factors except job experience. In the present study, the mean total flora colony count from the hands of HCWs before the hand hygiene procedure was undertaken showed that the hands of females were less contaminated than those of males. Concordant with this, when the difference between the mean number of total flora colonies before and after hand hygiene procedures was assessed, the mean reduction of the total flora colony count was greater in females than in males. These findings showed that female HCWs obeyed the hand hygiene rules more than males. A similar difference between nursing assistants and nurses was also observed: nurses' hands were less contaminated. The number of mean total flora on the hands of HCWs working in the paediatrics and newborn ward was lower than those of HCWs from other wards, indicating that HCWs in that ward obeyed hand hygiene rules more than the HCWs in other wards.

The effectiveness of hand hygiene as an infection control measure relates not only to the frequency with which it is carried out, but also to how effectively it is undertaken. Reductions in total and transient flora were measured in the present study in order to evaluate the effectiveness of the two hand hygiene techniques in a practical setting. There was no significant difference between the mean number of total flora colonies before and after hand hygiene with both techniques. After use of the HWR technique, however, the number of transient flora colonies was significantly reduced compared with the HW technique.

A reduction in the number of transient flora colonies is quite important in the prevention of nosocomial infections. Winnefeld *et al.*<sup>13</sup> compared non-antiseptic liquid soap use with alcohol-based disinfectant use and reported that alcohol-based rinse was significantly more effective than liquid soap in removing transient contaminant microorganisms. Several experimental studies, in which hands have been artificially contaminated with various microorganisms have shown hand rubbing with alcohol-based products to be more effective than hand washing with unmedicated or antiseptic soap.<sup>19–23</sup>

Considerable data from laboratory studies support the view that alcohol-based hand disinfectants are effective for the inactivation of most Gram-positive and Gram-negative bacteria, as well as fungi.<sup>5</sup> In an experimental study, hand washing did not prevent the transfer of aerobic Gram-negative bacilli by HCWs from heavily colonized patients to urinary catheters in all of 12 cases.<sup>24</sup> In contrast, after hand treatment with isopropyl alcohol, bacteria were transferred in only two of 12 cases.<sup>24</sup> Tvedt and Bukholm<sup>25</sup> reported that six of 10 *S. aureus* strains regrew after hand washing,

but only three after hand disinfection. They noted that hand disinfection was more effective at inactivating pathogenic bacteria than hand washing. In the present study, the HWR technique was determined to be significantly more effective than HW for the elimination of transient flora and was moderately effective against all Gram-negative and Gram-positive bacteria, except *S. aureus*.

According to Dharan *et al.*,<sup>26</sup> the bactericidal activity of ethanol against *S. aureus*, *Enterococcus faecium*, or *P. aeruginosa* seemed to be slightly higher at 80% concentration than at 95%. According to the 'tentative final monograph for healthcare antiseptic products',<sup>27</sup> ethanol is considered to be generally effective at between 60% and 95%, but the spectrum of bactericidal activity of ethanol is broad.<sup>5</sup> When assessed from this point of view, the 77% ethanol concentration in the disinfectant used in the present study may be regarded as effective.

In conclusion, the present study of two hand hygiene procedures, HW and HWR, and investigation of the numbers and types of microorganism isolated from the hands of HCWs based on demographic characteristics indicated that the hands of HCWs were contaminated with various Gram-positive and Gram-negative bacteria and yeast. Both hand hygiene procedures were effective in reducing numbers of total and transient flora colonies. The reduction of transient flora colony numbers, which is important for nosocomial infection control was, however, significantly greater following HWR than after HW. Elimination of Gram-positive bacteria, except *S. aureus*, Gram-negative bacteria and yeasts from the hands of HCWs after HWR was particularly reassuring as this is the hand hygiene procedure used routinely at Gaziantep University Hospital. Larger scale investigations on this topic should be



undertaken and the effectiveness of alcohol-based disinfectants against *S. aureus* should be improved.

## Conflicts of interest

The authors had no conflicts of interest to declare in relation to this article.

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