

Effectiveness of alcohol solutions at different concentrations as a disinfectant for hand hygiene

María de los Ángeles Lizbeth Muñiz Alvarado¹, Luis Enrique Pérez Gómez², Jesús Abraham Aguilar Campos², Porfirio Felipe Hernández Bautista³, Guadalupe García Bracamontes¹

1. Regional General Hospital No. 1, Ciudad Obregón 85110, Mexico.
2. High Specialty Medical Unit 08, Specialty Hospital, Ciudad Obregón 85120, Mexico.
3. Coordination of Quality of Supplies and Specialized Laboratories, Mexican Institute of Social Security, Mexico City 07760, Mexico.

Abstract

Introduction: Healthcare-associated infections are the leading cause of prolonged hospital stays, as well as leading cause of long-term disability, increased deaths, and increased costs. Hand hygiene has been a highly effective intervention in reducing healthcare-associated infections; however, the alcohol concentration range is very wide (60% to 95%), in line with WHO recommendations.

Objective: To evaluate the effectiveness of alcohol-based solutions at different concentrations as hand hygiene disinfectants for healthcare personnel and family members of patients at Regional General Hospital 1, Ciudad Obregón, Sonora.

Materials and methods: A quasi-experimental study was carried out which included health personnel working at the Regional General Hospital 1 and relatives of hospitalized patients in the different services (the caregiver), the hand hygiene technique was performed in vivo and in vitro to assess susceptibility according to the 5 most frequent microorganisms in Infections associated with health care and with a pattern of antimicrobial multi-resistance from March 1 to July 31, 2015.

Results: The reduction obtained with 90% alcohol solution was 97.79% at 10 seconds and 98.67% at 30 seconds, with 60% a reduction of 76.68% and 82.42% respectively; a greater reduction was observed with 90% alcohol solution and hand hygiene technique: 98.06% at 10 seconds and 99.20% at 30 seconds.

Conclusions: The 90% alcohol solution was the factor that showed statistical significance for both Gram positive and Gram-negative bacteria.

Keywords: Effectiveness, alcohol solutions, Healthcare-associated infections.

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INTRODUCTION

A healthcare-associated infection (HAI) is a localized or systemic condition, resulting from an infectious agent or its toxins, contracted by a patient during treatment in a hospital or other healthcare facility and which was neither present nor in the incubation phase at the time of admission.¹

Worldwide, it is estimated that more than 1.4 million people contract hospital-acquired infections; between 5% and 10% of patients admitted to hospitals in developed countries will contract one or more infections. In the United States of America (USA), one in every 136 hospitalized patients becomes seriously ill from a hospital-acquired infection, equivalent to 2 million cases and approximately 80,000 deaths annually.²

This generates an average cost ranging from \$382.00 to \$1,833.00 US per case; In Canada, the annual cost of nosocomial infections is \$0.3 to \$1 billion; in Germany, the annual cost is estimated at \$0.3 to \$0.6 billion.

HAIs are the leading cause of prolonged hospital stays, as well as long-term disability, increased deaths, and increased costs.⁴

Several studies have shown that poor hand hygiene is the most common cause of HAIs.^{5, 6} Similarly, it has been observed that handwashing with alcohol-based solutions prevents the transmission of nosocomial pathogens by healthcare workers, as alcohols possess antimicrobial activity attributed to their ability to denature proteins.^{7, 8}

Alcohol-based solutions, at concentrations of 60% to 95%, are more effective, while higher concentrations are less potent, as proteins do not denature easily in the absence of water. They also reduce hand hygiene time. They have been shown to have excellent in vitro germicidal activity against gram-positive and gram-negative bacteria, including multidrug-resistant pathogens, *Mycobacterium tuberculosis*, and several fungi. Some enveloped viruses, such as the Human Immunodeficiency Virus, Herpesvirus, Influenza, and Respiratory Syncytial Virus, are sensitive to alcohol.^{7, 8}

It is therefore important to practice proper hand hygiene to reduce healthcare-associated infections. Various guidelines recommend that handwashing be performed with alcohol-based solutions at a concentration of 60% for a period of 30 seconds to be effective. The World Health Organization even establishes guidelines for preparing alcohol-based solutions, thus ensuring greater effectiveness.^{9, 10, 11}

It is worth noting the rise in the use of alcohol-based solutions in recent years, as mentioned by Angeles et al. (2005) in their clinical trial conducted at the IMSS La Raza Medical Center (CMN la Raza), where they compared the effectiveness of using alcohol gel and the traditional hand disinfection technique in reducing CFUs on the hands of doctors and nurses, obtaining a CFU-Log10 reduction of 0.7 versus 1.53 ($p \leq 0.01$); observing that the nurses group had a greater reduction in CFUs-Log10 than the doctors ($p < 0.05$).

In 2004, at the Department of Medicine of the State University of Maringá, Brazil, Hernandez et al. compared the effectiveness of alcohol-based hand gel with traditional handwashing for the elimination of agents such as *Acinetobacter baumannii*, methylcylin-resistant *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Candida albicans*. They concluded that handwashing with liquid soap reduced the number of microorganisms by 93.83%, 100% povidone-iodine, and 99% alcohol-based hand gel. They also demonstrated that 4 of 6 microorganisms analyzed with the different disinfectants were removed.¹³

On the other hand, Alvarado et al. (2008) studied the antiseptic capacity of two different brands of alcohol-based hand gel, without specifying the final alcohol concentration, and compared its effectiveness with a 70% liquid alcohol solution. They found that the greatest antimicrobial effects for both gels were obtained after 30 s and for liquid alcohol after 1 minute. An ANOVA distribution was used for statistical analysis and it was concluded that the alcohol presentation does not interfere with the percentages of bacterial reduction and the time for maximum bacterial inhibition is 30 s. 14 Kampf and Hollingsworth (2008) evaluated the bactericidal activity of an ethanol-based hand gel in 15 s and its efficacy against 11 gram-positive bacteria, 16 gram-negative bacteria, and 11 emerging bacterial pathogens. Each strain was evaluated in quadruplicate; They found that hand gel (85% ethanol) reduced 11 gram-positive and 16 gram-negative bacteria by more than 5 logs in 15 seconds and that it has a broad bactericidal spectrum. 15 Herruzo-Cabrera et al. (2001) studied the effects of various alcohol solutions on the resident and acquired microbiota, comparing them with conventional handwashing in vivo and in vitro. Significant differences were found in both designs. Handwashing barely modified the native or acquired microbiota (only 0.1 to < 2 logs) and did not eliminate *Staphylococcus aureus* and gram-negative bacteria (34–23%; $P > 0.05$). However, N-duopropenide reduced the acquired microbiota by 5 logs and the native microbiota by more than 2 logs, as well as *S. aureus* and gram-negative bacteria (33–1.3%; $P < 0.01$).

MATERIALS AND METHODS

The study was conducted at Regional General Hospital No. 1, located in Ciudad Obregón, Sonora. The study period ran from March 1 to July 31, 2015. A quasi-experimental, double-blind study was conducted with healthcare personnel and family members of patients who met the inclusion criteria.

The sample size was calculated and yielded 52 participants. The study included healthcare personnel (physicians, nurses, and residents) working at the Northwest National Medical Center in Ciudad Obregón, Sonora, as well as family members of hospitalized patients (only the caregiver) from departments with the highest incidence of healthcare-associated infections (internal medicine, gynecology and obstetrics, surgery, traumatology, orthopedics, pediatrics, and emergency departments). Individuals with product allergies, dermatitis, prior hand hygiene, family members other than the patient's caregivers, or visibly dirty hands were excluded, and samples contaminated during transport or planting were eliminated.

The dependent variable was hand disinfection effectiveness, and the independent variables were the use of alcohol-based hand sanitizers at different concentrations, sex, adverse reactions, hand hygiene technique recommended by the WHO, hand rubbing time, assigned service, type of worker, and pathogen isolated.

The study protocol was submitted to the local research committee, and written consent was obtained from study participants.

The alcohol-based hand sanitizer was prepared at different concentrations (60% and 90%) according to the WHO-recommended guidelines for the preparation of hand sanitizer formulations. This was carried out as follows: 96% ethanol (8333 ml) was poured into a 10-liter plastic bottle fitted with a screw-top cap, 3% hydrogen peroxide (417 ml) was added, and 98% glycerol (145 ml) was added using a measuring cylinder. The bottle was then filled to the 10-liter mark with sterile distilled water. Once the preparation was completed, it was immediately capped to prevent evaporation. The solution was mixed by gentle shaking, and the solution was distributed into 100-ml plastic containers. The bottles were quarantined for 72 hours to eliminate any spores present in the alcohol or bottles. Quality control was monitored using an alcohol breathalyzer.

Initially, a hand sample was taken with a swab moistened with 0.45% saline solution, rubbing it over the entire surface. The swab was then placed in Brain-

Heart Infusion (BHI) broth. Subsequently, 2 ml of alcohol-based solution at 60% and 90% concentrations was applied to the hands, and hands were washed. Most healthcare personnel performed this procedure using the WHO-recommended technique, while patient family members did not. Samples were taken again 10 and 30 seconds after application. The collected samples were taken to the laboratory, incubated for 4 hours, and cultured using the mass plating technique with a 0.1 ml calibrated loop in base agar for CFU counting. For microorganism identification, samples were plated on blood agar, MacConkey agar, and mannitol salt agar. They were kept in incubation for 24 hours, at the end the colonies were isolated for identification by direct observation on a plate, as well as observation with a microscope and if necessary they were introduced to the VITEK 2. As a complement to the study, in vitro susceptibility tests were performed with a suspension of bacteria in a test tube with 0.45% saline solution as a diluent, it was verified that it had an optical density of 0.5 McFarland and dilutions 1:2, 1:4, 1:8, 1:16 and 1:32 were made with the different concentrations of alcohol solution (2 ml), the sample was homogenized by vortexing and the inhibition of strains was evaluated at 10s and 30s, a sample was taken with the calibrated loop extended on the plate, it was left to incubate for 24 hours and the CFU count was performed, the microorganisms were selected according to their development in cultures of hospitalized patients, the five most common or outbreak-causing pathogens (*Acinetobacter baumannii* complex, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia*) were identified, and the pattern of antimicrobial multidrug resistance was established.

A form was created in Epi Info 7® for all participants, including healthcare personnel and family members of hospitalized patients. A univariate analysis was then performed, applying frequency and central tendency measures depending on the type of variable, as well as proportions for qualitative variables.

Quantitative variables were evaluated using ANOVA statistics. A bivariate analysis was also performed using the Mann-Whitney U test. For qualitative variables, the chi-square test was used. Risk assessment was performed using Hazard Ratio. Multivariate analysis was performed using Cox proportional hazards. The software used was STATA® and Epi Info 7®.

RESULTS

Data was collected from a total of 132 participants of both sexes over a five-month period. The study participants were 31.06% male and 68.93% female. The categories included were attending physicians (13.63%), resident physicians (23.48%), nurses (40.15%), and patient family members (caregivers) (22.72%). The alcohol-based hand sanitizer was applied in equal proportions to each study subject. Of the total participants, 71.96% performed hand hygiene using the WHO-recommended technique, and 28.03% did not (Table 1). According to microorganism development, 18 Gram-positive strains (64.28%) and 10 Gram-negative strains (35.71%) were isolated, totaling 171 bacteria. Among these strains, coagulase-negative Staphylococcus (20.46%) were isolated in order of frequency, followed by Acinetobacter baumannii complex (18.12%), Staphylococcus haemolyticus (8.77%), Staphylococcus hominis (8.77%), and Staphylococcus warneri (7.60%) (Table 1).

Table 1. General characteristics of health personnel and patient caregiver and most frequent microorganisms

Variable	Number	Percentage
Gender		
Male	41	31.06%
Female	91	68.93%
Category		
Primary care physicians	18	13.63%
Resident physicians	31	23.48%
Nurse	53	40.15%
Patient caregiver	30	22.72%
Hand hygiene		
with technique	95	71.96%
without technique	37	28.03%
Gram positive microorganisms	18	64.28%
Gram negative microorganisms	10	35.71%
Most common microorganisms		
Staphylococcus coagulasa negativo	35	20.46%
Acinetobacter baumannii complex	31	18.12%
Staphylococcus haemolyticus	15	8.77%
Staphylococcus hominis	15	8.77%
Staphylococcus warneri	13	7.60%

Source: Analysis of information obtained at the Regional General Hospital 1, Obregon, Sonora.

The reduction obtained with the 90% alcohol solution was 97.79% at 10 s and 98.67% at 30 s. The reduction obtained with the 60% solution was 76.68% and 82.42%, respectively. A greater reduction was observed with 90% alcohol solution using hand hygiene: 98.06% at 10 s and 99.20% at 30 s (Table 2).

Table 2. CFU inhibition at 10s and 30s

Variable	CFU Previous/Mean	CFU 10s	CFU 30s
90% alcohol solution	226.61	97.79%	98.67%
60% alcohol solution	238.09	61.75%	82.42%
Hand hygiene with technique	202.49	76.68%	85.96%
Hand hygiene without technique	307.4	83.26%	97.48%
90% alcohol solutions with hand hygiene technique	200.44	98.06%	99.20%
90% alcohol solutions without hand hygiene technique	294.94	97.16%	97.45%
60% alcohol solutions with hand hygiene technique	204.58	54.81%	73.28%
60% alcohol solutions without hand hygiene technique	319.21	72.51%	97.51%

Proportions at 10s and 30s. CFU: Colony Forming Units, s: seconds.

Source: Analysis of information obtained at the Regional General Hospital 1, Obregon, Sonora.

Colony-forming unit inhibition was evaluated using analysis of variance (ANOVA), which compared the inhibition with 90% alcohol solution, obtaining a $p < 0.001$, with a mean of 226.61 CFU in the pre-culture, with development of 5 CFU at 10 s and 3.01 CFU at 30 s after application of the alcohol solution. When applied with 60% alcohol solution, a $p = 0.03$ was obtained, with a mean of 238.09 CFU in the pre-culture, 91.6 CFU at 10 s, and 41.85 CFU at 30 s (Table 3).

Table 3. Inhibition of CFU dependent on alcohol solution and hand hygiene technique

Variable	CFU Previous/Mean	CFU 10s/Mean	CFU 30s/Mean	p value
90% alcohol solution	226.61	5	3.01	<0.001
60% alcohol solution	238.09	91.06	41.85	0.003
Hand hygiene with technique	202.49	47.21	28.42	0.001
Hand hygiene without technique	307.4	51.45	7.74	0.001
90% alcohol solutions with hand hygiene technique	200.44	3.87	1.59	0.001
90% alcohol solutions without hand hygiene technique	294.94	8.37	7.5	0.001
60% alcohol solutions with hand hygiene technique	204.58	92.43	54.66	0.077
60% alcohol solutions without hand hygiene technique	319.21	87.73	7.94	0.032

P values calculated using ANOVA; CFU at 10 and 30 seconds were compared with the previous CFU. CFU: Colony Forming Units, s:seconds

Source: Analysis of information obtained at the Regional General Hospital 1, Obregon, Sonora.

Regarding hand hygiene with the technique, a $p < 0.001$ was observed, with a mean of 202.49 CFU before, 47.21 CFU after 10 seconds, and 28.42 CFU after 30 seconds. When hand hygiene was performed without the technique, a $p < 0.001$ was observed, with a mean of 307.40 CFU before, 51.45 CFU after 10 seconds, and 7.74 CFU after 30 seconds (Table 3). Regarding the use of 90% alcohol solution with hand hygiene technique, a $p < 0.001$ was found with a mean of 200.44 in the previous, 3.87 at 10s and 1.59 at 30s, the same solution, but without technique presented a $p < 0.001$, with 294.94 CFU in the previous, 8.37 at 10s and 7.5 at 30s; A difference was noted with the 60% alcohol solution with the technique, as it presented a $p=0.07$, not being statistically significant with a mean reduction of 92.43 CFU at 10 s and 54.66 CFU at 30 s. However, without the technique, a $p=0.03$ was obtained, with a

mean reduction of 87.73 at 10 s and 7.94 CFU at 30 s from a previous development of 319.21 CFU .

Table 4. Reduction of microorganisms at 10s and 30s

Variable	HR* 10s	95% CI	p value	HR* 30s	95% CI	p value
90% alcohol solution	0.62	0.47-0.82	0.004	0.57	0.39-0.84	0.002
Hand hygiene technique	1.02	0.76-1.37	0.87	0.86	0.60-1.24	0.447
Category						
Nurse	1.01	0.78-1.30	0.927	0.86	0.60-1.23	0.417
Resident physician	1.02	0.77-1.36	0.868	0.85	0.55-1.31	0.442
Gram negative	1.19	0.93-1.52	0.189	1.31	0.93-1.83	0.135

95% CI: 95% Confidence Interval/Statistically significant p-value <0.05. Risk was assessed using HR: Hazard Ratio, s=seconds.

Source: Analysis of information obtained at the Regional General Hospital 1, Obregon, Sonora.

The risk was evaluated through Hazard Ratio (HR), observing that the application of 90% alcohol solution represents a protective factor for the development of microorganisms at 10s (HR 0.62; 95% CI, 0.47-0.82; $p < 0.001$), with no difference with the hand hygiene technique. (HR 1.02; 95% CI, 0.76-1.37; $p = 0.87$) a comparison was made between nurses and resident physicians, without showing differences between them (HR 1.01; 95% CI, 0.78-1.30; $p = 0.92$) and (HR 1.02; 95% CI, 0.77-1.36; $p = 0.86$), finally Gram-negative microorganisms were evaluated (HR 1.19; 95% CI, 0.93-1.52; $p = 0.18$) (Table 4). The same was done at 30s, and again, the protective factor was found to be 90% alcohol solution (HR 0.57; 95% CI, 0.39-0.84; $p < 0.001$), hand hygiene technique (HR 0.86; 95% CI, 0.60-1.24; $p = 0.44$), category (nurses and medical residents) (HR 0.86; 95% CI, 0.60-1.23; $p = 0.41$) and (HR 0.85; 95% CI, 0.55-1.31; $p = 0.44$). There was no difference in Gram-negative bacteria (HR 1.31; 95% CI, 0.93-1.83; $p = 0.13$) (Table 4). In the analysis by microorganisms a difference was observed with alcoholic solution and hand hygiene technique mainly, as in the case of *Acinetobacter baumannii* complex with 90% and 60% alcoholic solution (previous CFU/mean 272.91; CFU 10s/mean 6.33; CFU 30s/mean 4.5; $p < 0.001$), (previous CFU/mean 236.93; CFU 10s/mean 50.06 CFU 30s/mean 7.85; $p = 0.01$) with and without hand hygiene technique (previous CFU/mean 258.90; CFU 10s/mean 19.8; CFU 30s/mean 5.42; $p < 0.001$), (previous CFU/mean 418.8; CFU 10s/mean 51.5 CFU 30s/mean 25; $p = 0.03$), having a similar behavior in the case of Gram positives (Tables 5-9).

Table 5. Coagulase-negative Staphylococcus isolated from the hands of healthcare personnel and patient caregivers

Variable	CFU	CFU	CFU	p value
	Previous/Mean	10"/Mean	30"/Mean	
90% alcohol solution	209.77	5.27	2	0.007
60% alcohol solution	253.07	36.61	10	0.015
Hand hygiene with technique	214.26	12.57	6.15	<0.001
Hand hygiene without technique	259.33	29.44	0.28	0.05
90% alcohol solutions with hand hygiene technique	215.93	7	2.5	0.001
90% alcohol solutions without hand hygiene technique	193.33	0.66	0.4	0.037
60% alcohol solutions with hand hygiene technique	211.6	21.5	12	0.025
60% alcohol solutions without hand hygiene technique	391.33	87	0	0.536

P values calculated using ANOVA; CFU at 10 and 30 seconds were compared with the previous CFU. Most common microorganism. CFU: Colony Forming Units, s = seconds

Source: Analysis of information obtained at the Regional General Hospital 1, Obregon, Sonora.

Tabla 6. Acinetobacter baumannii complex aislado en manos de personal de salud y cuidadores de pacientes

Variable	CFU	CFU	CFU	p value
	Preview/Mean	10"/Mean	30"/Mean	
90% alcohol solution	272.91	6.33	4.5	0.002
60% alcohol solution	236.93	50.06	7.85	0.01
Hand hygiene with technique	258.9	19.8	5.42	<0.001
Hand hygiene without technique	418.8	51.5	25	0.038
90% alcohol solutions with hand hygiene technique	325.7	7.6	5.4	0.001
90% alcohol solutions without hand hygiene technique	466.4	0.25	0	0.15
60% alcohol solutions with hand hygiene technique	198.18	30.9	5.45	0.019
60% alcohol solutions without hand hygiene technique	343.5	102.75	16.66	0.406

Valores de p calculados mediante ANOVA; se comparo UFC a los 10s y 30s con UFC previo. UFC: Unidades Formadoras de Colonias, s=segundos.

Source: Analysis of information obtained at the Regional General Hospital 1, Obregon, Sonora.

Tabla 7. Staphylococcus haemolyticus isolated from the hands of healthcare personnel and patient caregivers.

Variable	CFU	CFU	CFU	p value
	Preview/Mean	10"/Mean	30"/Mean	
90% alcohol solution	170.66	4.66	0	0.09
60% alcohol solution	317.33	27.33	10.83	0.093
Hand hygiene with technique	150.1	7.5	0.4	0.035
Hand hygiene without technique	387.8	26.2	12.2	0.128
90% alcohol solutions with hand hygiene technique	116.5	2	0	0.298
90% alcohol solutions without hand hygiene technique	279	10	0	0.39
60% alcohol solutions with hand hygiene technique	200.5	15.75	1	0.143
60% alcohol solutions without hand hygiene technique	551	50.5	30.5	0.507

P values calculated using ANOVA; CFU at 10 and 30 seconds were compared with the previous CFU. CFU: Colony Forming Units, s = seconds.

Source: Analysis of information obtained at the Regional General Hospital 1, Obregon, Sonora.

Tabla 8. Staphylococcus hominis isolated from the hands of healthcare personnel and patient caregivers

Variable	CFU	CFU	CFU	p value
	Preview/Mean	10"/Mean	30"/Mean	
90% alcohol solution	516.85	3.14	2.33	0.147
60% alcohol solution	294.5	18.83	5.16	0.077
Hand hygiene with technique	466.44	14.5	5.14	0.082
Hand hygiene without technique	247.4	3.8	1.8	0.233
90% alcohol solutions with hand hygiene technique	688	4.4	3.5	0.176
90% alcohol solutions without hand hygiene technique	89	0	0	0.445
60% alcohol solutions with hand hygiene technique	189.5	31.33	7.33	0.281
60% alcohol solutions without hand hygiene technique	353	6.33	3	0.375

P values calculated using ANOVA; CFU at 10 and 30 seconds were compared with the previous CFU. CFU: Colony Forming Units, s = seconds..

Source: Analysis of information obtained at the Regional General Hospital 1, Obregon, Sonora.

Tabla 9. Staphylococcus warneri aislado en manos de personal de salud y cuidadores de pacientes

Variable	CFU	CFU	CFU	p value
	Preview/Mean	10"/Mean	30"/Mean	
90% alcohol solution	58.33	4	0	0.01
60% alcohol solution	51.28	21.14	6.14	0.277
Hand hygiene with technique	77.37	19.87	5	0.016
Hand hygiene without technique	18	2.6	0.6	0.271
90% alcohol solutions with hand hygiene technique	55.6	4.8	0	0.051
90% alcohol solutions without hand hygiene technique	113.66	45	13.33	0.225
60% alcohol solutions with hand hygiene technique
60% alcohol solutions without hand hygiene technique	4.5	3.25	0.75	0.241

Valores de p calculados mediante ANOVA; se comparo UFC a los 10s y 30s con UFC previo. UFC: Unidades Formadoras de Colonias, s=segundos.

Source: Analysis of information obtained at the Regional General Hospital 1, Obregon, Sonora.

A difference was observed in the Mann-Whitney U test regarding the use of alcohol-based hand rub ($p < 0.001$ at 10 s; $p < 0.001$ at 30 s) and the combination of alcohol-based hand rub with and without hand hygiene ($p = 0.003$; $p = 0.039$, respectively). This was not the case for the hand hygiene technique, where no difference was observed ($p = 0.356$) (Table 10).

Table 10. Reduction of CFU at 10s and 30s with alcohol solution and hand hygiene technique.

Variable	CFU		p value	CFU	
	Preview/Median	10s/Median		30s/Median	p value
90% alcohol solution	63.22	50.77	<0.001	51.03	0.001
60% alcohol solution	67.78	79.01		69.35	
Hand hygiene with technique	65.36	64.65	0.852	59.05	0.356
Hand hygiene without technique	65.85	65.94		64.66	
90% alcohol solutions with hand hygiene technique	41.47	36.17	0.001	37.88	0.003
90% alcohol solutions without hand hygiene technique	47.53	52.83		51.13	
60% alcohol solutions with hand hygiene technique	13.65	11.27	0.02	11.69	0.039
60% alcohol solutions without hand hygiene technique	16.91	18.74		18.41	

p values calculated using Mann-Whitney U; CFU: Colony Forming Units, s: seconds. Sol: solution.

Source: Analysis of information obtained at the Regional General Hospital 1, Obregon, Sonora.

A chi-square test was performed to determine differences in the microbiota developed by healthcare personnel and patient caregivers. No differences in the development of microorganisms were observed with this method ($p=0.10$).

In the multivariate analysis performed using Cox proportional hazards, it was observed that the 90% alcohol solution reduced the bacterial load up to twofold more when rubbed for 30 s (HR of 2.13; 95% CI, 1.19-3.81; $p = 0.01$), while the hand hygiene technique had no association (HR 1.08; 95% CI, 0.55-2.12; $p = 0.81$) and Gram-negative bacteria were also not statistically significant (HR 0.67; 95% CI, 0.34-1.32; $p = 0.25$) (Table 11). When analyzing the CFU reduction at 30 s, a difference was only observed with the alcohol solution, with a HR of 2.42; 95% CI, 1.50-3.89; $p < 0.001$. This difference was corroborated in the multivariate analysis. No differences were observed for the remaining variables (Table 12).

Table 11. Multivariate analysis of the reduction of microorganisms at 30s

Variable	HR*	95%CI	p value
90% alcohol solution	2.13	1.19-3.81	0.011
Hand hygiene technique	1.08	0.55-2.12	0.81
Gram negative	0.67	0.34-1.32	0.259

Cox proportional hazards. 95% CI: 95% confidence interval. Statistically significant p-value <0.05. s: seconds.

Source: Analysis of information obtained at the Regional General Hospital 1, Obregon, Sonora.

Table 12. Multivariate analysis of the reduction of Colony Forming Units at 30s

Variable	HR*	95%CI	p value
90% alcohol solution	2.42	1.50-3.89	<0.001
Hand hygiene technique	1.14	0.66-1.96	0.636
Gram negative	0.86	0.52-1.44	0.586

Cox proportional hazards. 95% CI: 95% confidence interval. Statistically significant p-value <0.05. HR: Hazard ratio, s:seconds.

Source: Analysis of information obtained at the Regional General Hospital 1, Obregon, Sonora.

It is worth mentioning that contaminated samples were excluded from the analysis. In vitro tests performed with the five main microorganisms that developed at Regional General Hospital 1 with a pattern of antimicrobial multidrug resistance, a significant reduction in CFU was observed with the 90% alcohol solution, which was greater at 30 s in most dilutions. This was the case with the 60% alcohol solution, where a smaller reduction was observed, especially in microorganisms such as *Klebsiella pneumoniae* and *Acinetobacter baumannii* complex (Table 13).

DISCUSSION.

The presence of HAIs represents a serious problem for patient safety, and their prevention should be a priority in healthcare institutions.³

The main objective of this study was to evaluate the effectiveness of alcohol-based solutions at different concentrations as a disinfectant for hand hygiene among healthcare personnel and patients' families. This was achieved by observing a reduction in CFUs with the use of 60% and 90% alcohol-based solutions. The latter was more effective, achieving a greater reduction within 30 seconds. This is in agreement with Kampf G and Ostermeyer C., who demonstrated the efficacy of alcohol-based solutions at concentrations between 60% and 95%, conferring efficacy against Gram-positive and Gram-negative bacteria.⁷

Similarly, a considerable reduction in CFU was observed when performing hand hygiene in 10 s, which is slightly lower than the WHO recommendation and the time observed in the Kampf and Hollingsworth study, which evaluated the bactericidal activity of an ethanol-based hand gel in 15 s, finding efficacy against 11 gram-positive bacteria, 16 gram-negative bacteria, and 11 emerging bacterial pathogens.¹⁵ Therefore, hand hygiene is the main measure whose effectiveness in preventing HAIs has been demonstrated. Such is the case of Angeles et al. in their clinical trial, which demonstrated the effectiveness of using alcohol gel for hand disinfection by reducing CFUs in the hands of doctors and nurses, obtaining a Log₁₀ CFU reduction of 1.53 ($p < 0.01$). They observed that the nurses had a greater Log₁₀ CFU reduction than the doctors ($p \leq 0.05$), which is consistent with the results of this study regarding CFU reduction. However, there is a discrepancy regarding the reduction in the categories since no difference was observed between them.¹²

In the bivariate analysis, a difference was observed with the alcohol-based hand sanitizer, but this was not the case for the hand hygiene technique.

In the multivariate analysis, a difference was observed only with the alcohol-based hand sanitizer, both for the reduction of microorganisms (HR: 2.13; 95% CI, 1.19–3.81) and for Colony-Forming Units (HR: 2.42; 95% CI, 1.50–3.89).

In this study, a 97.79% reduction was observed at 10 seconds, with a greater reduction achieved at 30 seconds, reaching 98.67% with the 90% alcohol-based hand sanitizer. This correlates with the results of Hernandez in Brazil, who compared the effectiveness of alcohol-based hand sanitizer with traditional handwashing. They concluded that the alcohol-based hand sanitizer reduced the risk of up to 99% and significantly removed Gram-negative bacteria. There are studies such as that of Alvarado et al., which investigated the antiseptic properties of alcohol gel without specifying the concentration and 70% liquid alcohol. They found a greater effect with alcohol gel after 30 seconds and with liquid alcohol up to one minute after application. This aspect is not consistent with this study, since a significant reduction was observed after 10 seconds; however, this could have been due to the lower ethanol concentration than the one used in this study.¹⁴

In vitro tests, a greater reduction in CFU was observed with the 90% alcohol solution than with the 60% solution, which is consistent with what Herruzo-Cabrera demonstrated in the comparative study of conventional handwashing and alcohol-based solutions in vivo and in vitro, where a greater reduction in CFU was found with the latter, including Gram-negative microorganisms. The use of alcohol gel offers several benefits, including faster hand hygiene, fewer adverse reactions (which occurred in only 2% of participants in this study), immediate availability at the patient's bedside, and lower cost.

This work provides a basis for future research to evaluate antimicrobial resistance and sensitivity *in vivo* hand hygiene studies, as well as serving as a basis for reconsidering proper hand hygiene.

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