Monitoring the effectiveness of surgical handwashing in healthcare workers of a fourth level operating room using bioluminescence assays

Monitoreo de la eficacia del lavado de manos quirúrgico en el personal de salud de un quirófano de cuarto nivel mediante ensayos de bioluminiscencia

Monitorando a eficácia da lavagem cirúrgica das mãos em profissionais de saúde em uma sala de cirurgia de quarto nível usando ensaios de bioluminescência

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Received: June 17th, 2022
Accepted: August 30th, 2022
Available: September 12th, 2022

How to cite this article:
C. M. Ramírez Espinosa, I.C. Guayan Ardila, D.P. Torres Gil, J.Y. Serrano Riaño, J.J. Vaca-González, "Monitoring the Effectiveness of Surgical Handwashing in Healthcare Workers of a Fourth Level Operating Room Using Bioluminescence Assays," *Revista Ingeniería Solidaria*, vol. 18, no. 3, 2022. doi: https://doi.org/10.16925/2357-6014.2022.03.08

Research article. https://doi.org/10.16925/2357-6014.2022.03.08

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Abstract

This article is a product of the project "Effectiveness of the handwashing technique in workers of the surgical team at the Hospital Universitario Clínica San Rafael in the year 2021", developed by Fundación Universitaria del Área Andina and Hospital Universitario Clínica San Rafael.

Aim: To assesses the effectiveness of surgical handwashing (S-HW) in healthcare workers (HCWs) in terms of organic material and bacteria elimination by means of bioluminescence assays.

Methods: 62-HCWs were subjected to bioluminescence analyses to determine adenosine triphosphate (ATP) and colony forming units (CFU) of *Enterobacteriaceae* (EB) and *Escherichia coli* (*E. coli*) before and after S-HW. A checklist to evaluate adherence to hand hygiene (HH) protocol was implemented.

Results: ATP before HH (510.9/RLU) was reduced after S-HW (54.6/RLU), evidencing a significant decrease (p=0.001). Regarding the bacteria, 26.6/CFU and 4/CFU for EB and *E. coli* were reported before S-HW, respectively. After S-HW, CFU decreased for EB (21.8/CFU) and *E. coli* (3.5/CFU) without showing a significant reduction (p=0.679). Low ATP did not evidence a direct relation with EB (p=0.082) and *E. coli* (p=0.680) reduction. Surgical instrument professionals (p=0.001), general surgeons (p=0.001), residents (p=0.017), orthopedists (p=0.029) and otolaryngologists (p=0.029) evidenced a reduction in ATP after S-HW. Factors such as S-HW direction, surgical soap, number of times and time of S-HW implemented showed a statistically significant difference in reduction of ATP, but not in CFU.

Conclusion: Although there was a reduction in elimination of ATP, there was no evidence of a decrease in bacteria. This finding not only helped identify potential errors in S-HW processes, but also led to the generation of strategies to improve HH to prevent healthcare-associated infections.

Limitation of the study: It was not possible to analyze other kinds of bacteria present on HCWs' hands such as *S. aureus, Pseudomonas aeruginosa, Klebsiella*, among others.

Keywords: Handwashing, Operating room, Healthcare worker, Adenosine triphosphate, Bacteria, Bioluminescence.

Resumen

Este artículo es producto del proyecto "Efectividad de la técnica de lavado de manos en trabajadores del equipo quirúrgico del Hospital Universitario Clínica San Rafael en el año 2021", desarrollado por la Fundación Universitaria del Área Andina y el Hospital Universitario Clínica San Rafael.

Objetivo: Evaluar la efectividad del lavado de manos quirúrgico (LVM-Q) en trabajadores de salud (TS) en términos de eliminación de material orgánico y bacterias mediante bioluminiscencia.

Métodos: 62-TS se sometieron a análisis de bioluminiscencia para determinar el trifosfato de adenosina (ATP) y unidades formadoras de colonia (UFC) de *Enterobacteriaceae* (*EB*) y *Escherichia coli* (*E. coli*) antes y después del LVM-Q. Se implementó una lista de verificación para evaluar la adherencia al protocolo de higiene de manos (HM).

Resultados: ATP antes de la HM (510.9/URL) se redujo significativamente después del LVM-Q (54.6/URL) (*p=0,001*). En cuanto a bacterias, hubo 26.6/UFC y 4/UFC para *EB* y *E. coli* antes del LVM-Q, respectivamente. Después del LVM-Q, las UFC disminuyeron para *EB* (21.8/UFC) y *E. coli* (3.5/UFC) sin evidencia significativa (*p=0.679*). Los niveles bajos de ATP no evidenciaron una relación directa con la reducción de *EB* (*p=0.082*) y *E. coli* (*p=0.680*). Profesionales de instrumentación quirúrgica (*p=0.001*), cirujanos (*p=0.001*), residentes (*p=0.017*), ortopedistas (*p=0.029*) y otorrinolaringólogos (*p=0.029*) evidenciaron reducción de ATP después del LVM-Q. Factores como dirección del LVM-Q, jabón quirúrgico, momentos y tiempo de LVM-Q mostraron una reducción significativa en ATP, pero no en UFC.

Conclusión: Aunque hubo reducción en la eliminación de ATP, no se evidenció una disminución en bacterias. Este hallazgo permitió identificar posibles errores en los procesos del LVM-Q y generar estrategias para mejorar la HM para prevenir infecciones asociadas a la atención de la salud.

Limitación del estudio: No fue posible analizar bacterias presentes en las manos de los TS como *S. aureus, Pseudomonas aeruginosa, Klebsiella*, entre otras.

Palabras clave: Lavado de manos, Quirófano, Trabajador de la salud, Trifosfato de Adenosina, Bacterias, Bioluminiscencia.

Resumo

Este artigo é produto do projeto "Eficácia da técnica de lavagem das mãos em trabalhadores da equipe cirúrgica do Hospital Universitário San Rafael Clínica em 2021", desenvolvido pela Fundação Universidade Área Andina e o Hospital Universitário San Rafael Clínica.

Objetivo: Avaliar a eficácia da lavagem cirúrgica das mãos (LVM-Q) em trabalhadores da saúde (HS) quanto à eliminação de matéria orgânica e bactérias por bioluminescência.

Métodos: 62-TS foi submetido à análise de bioluminescência para determinar trifosfato de adenosina (ATP) e unidades formadoras de colônias (UFC) de Enterobacteriaceae (EB) e Escherichia coli (E. coli) antes e depois do LVM-Q. Foi implementada uma lista de verificação para avaliar a adesão ao protocolo de higiene das mãos (HM).

Resultados: ATP antes do HM (510,9/URL) foi significativamente reduzido após LVM-Q (54,6/URL) (p=0,001). Em relação às bactérias, foram 26,6/UFC e 4/UFC para EB e E. coli antes do LVM-Q, respectivamente. Após o LVM-Q, as UFCs diminuíram para EB (21,8/UFC) e E. coli (3,5/UFC) sem evidência significativa (p=0,679). Baixos níveis de ATP não apresentaram relação direta com a redução de EB (p=0,082) e E. coli (p=0,680). Profissionais de instrumentação cirúrgica (p=0,001), cirurgiões (p=0,001), residentes (p=0,017), ortopedistas (p=0,029) e otorrinolaringologistas (p=0,029) evidenciaram redução do ATP após LVM-Q. Fatores como direção do LVM-Q, sabonete cirúrgico, momentos e tempo do LVM-Q mostraram redução significativa no ATP, mas não no CFU.

Conclusão: Embora tenha havido redução na eliminação de ATP, não houve evidência de diminuição de bactérias. Esse achado possibilitou identificar possíveis erros nos processos do LVM-Q e gerar estratégias para melhorar a HM para prevenir infecções associadas à assistência à saúde.

Limitação do estudo: Não foi possível analisar bactérias presentes nas mãos das profissionais do sexo como S. aureus, Pseudomonas aeruginosa, Klebsiella, entre outras.

Palavras-chave: Lavagem das mãos, Bloco operatório, Profissionais de saúde, Adenosina trifosfato, Bactérias, Bioluminescência.

1. INTRODUCTION

Hand hygiene (HH) has become very common over the last two years as one of the best defenses against COVID-19 [1], [2]; nevertheless, HH has been widely used for several years to prevent both healthcare-associated infections (HAIs) and transmission of multidrug-resistance microorganisms (MDROs) [3]. Even though the handwashing

(HW) process has been promoted as one of the most efficient and cost-effective strategies to reduce HAIs, it has been reported that compliance with HH among healthcare workers (HCWs) is low in both developed and developing countries [4], [5]. A study assessed the knowledge-perception of HH in 289 HCWs. Results evidenced that HH compliance improved significantly in pediatrics, internal medicine and obstetrics-gynecology after interventions. Additionally, in this study the perception of HCWs, in departments with interventions regarding the average percentage of hospitalized patients who will develop a HAI, increased significantly from 49.7 to 58.6% in the post-intervention phase [6]. A similar study identified that 72% of the units had no poster or written policy on HH, 87% did not have alcohol-based hand rubs, 98% had at least one handwash sink, 28% had flowing tap water all day, while 72% utilized cup and bucket and 58% had no hand drying facilities; moreover, results regarding the HCWs evidenced that HW was carried out by 21% before patient contact, 23% before aseptic procedure, 63% after body fluid exposure risk, 41% after contact with patients [7]. A study performed by Genc et al., determined the rate of nasal *S. aureus* carriage and methicillin resistance *S. aureus* (MRSA) in HCWs and evaluated the relationship between carriage and personal risk factors and HH behaviors. Results evidenced that the prevalence of *S. aureus* carriage was 20.1% and among 54 *S. aureus* carriers; only one person had MRSA (0.37%). However, *S. aureus* culture positivity was found to decrease significantly with increasing HW frequency [8]. Another study evidenced that HW compliance improved from 21.5% in 2010 to 75.1% in 2018; in fact, the HAI-incidence decreased significantly from 1.10 episodes per 1000 patient-days in 2010 to 0.45 per 1000 patient-days in 2018 [9].

Several techniques have been carried out in monitoring HH and effectiveness of hospital cleaning [10], where the most used are microbiological assays, fluorescent markers or adenosine triphosphate (ATP) bioluminescence tests. The latter seems to provide interesting features, such as the detection of ATP on surfaces (as RLU), which is a proxy of organic material and microbial contamination [11]. A study was conducted to evaluate cleaning/disinfection procedures in healthcare facilities, elucidating that ATP values below 50 RLU/cm^2 showed a reduction in samples above thresholds from 76% to 13% in the low-risk areas after disinfection [12]. Similarly, in the work developed by Boyce et al., ATP bioluminescence was implemented to assess the efficacy of daily hospital cleaning practices, identifying that bathroom grab bars and toilet seats were significantly cleaner after daily cleaning. Moreover, a total of 1013 ATP readings were obtained before and after daily cleaning in 105 rooms, showing that the median RLU was significantly lower after cleaning [13]. Similarly, a study evidenced that several surfaces were contaminated (>250 RLU), with the exception of incubator edges, where only 36 surfaces (30.5%) were considered clean. The highest median RLUs were obtained from telephone handset, light switches and blood pressure cuffs; however, significant improvements were obtained after educational intervention, as 115 surfaces (97.45%) were considered clean [14]. ATP tests have been also used to evaluate HH in HCWs; for instance, a study carried out in health personnel from a central sterile services department indicated that ATP levels before HW were 1687 RLU and after HH were below 98 RLU, where values <100 RLU were acceptable [15]. A study conducted with healthcare students evidenced that ATP levels before and after performing HW were 2951±40 URL vs. 400.7±73 URL, respectively [16]. It is relevant to mention that RLU values were not lower as expected, since RLU below 100 indicate clean hands. Another study performed with HCWs showed that RLU readings on arriving at hospital (320), after morning shift meeting (247) and during operation (143) were higher; however, average RLU readings decreased from 148 to 45 after a 3 month observation period [17]. A comparative study evaluated HH between neurosurgery and vascular surgery specialists. Results evidenced that ATP levels in both groups before HW were 20.276 ± 37.593 RLU, and after HW were 4190.5 ± 9062.7 RLU and 1328.5 ± 1478.8 RLU for those who used chlorhexidine gluconate and plain soap, respectively [18].

In accordance with the aforementioned, HW is a crucial practice to reduce not only HAIs, but also decrease organic material and microbial contamination on HCW hands. Although several studies have evaluated the compliance of HW steps on HCWs, there is a need to assess the adherence that surgical healthcare workers (S-HCWs) have with the S-HW protocols established by the World Health Organization (WHO). For this reason, the aim of this work was to assess the effectiveness of S-HW in health personnel who work in a fourth level operating room. Here, the S-HW process was evaluated throughout a checklist to identify the S-HW technique applied by HCWs. Additionally, bioluminescence assays were implemented to analyze the efficiency of the S-HW in the elimination of organic material and bacteria.

2. MATERIALS AND METHODS

2.1. Study design and participants

A quantitative and cross-sectional study was conducted in the Hospital Universitario Clínica San Rafael. This hospital-based study was reviewed by the hospital ethics committee throughout the CEI-122-2021 endorsement. Different studies, focused on assessing microbiological samples from hands of S-HCWs, who practice HW, were taken as the basis for the calculation of the sample for this study. For instance, in the study carried out by Storti et al., microbiological samples were taken from a total of 108 doctors, nurses and health aides [19]. Similarly, in the study developed by Sakita et al, microbiological samples of 54 HCW hands were analyzed [20]. According to the information provided by the General Surgery Department at Hospital Universitario Clínica San Rafael, there is a population of 112 workers belonging to the surgical area, distributed among residents, surgical instrument professionals and specialists. Based on this information, sample size was calculated with a 95% confidence interval and 5% error, resulting in 88 workers from the surgical area to be involved in the study.

2.2. Eligibility criteria

S-HCWs who met the following criteria were included in the study: 1) HCWs who perform S-HW, specifically, surgical instrument professionals, residents, and specialists, 2) S-HCWs must belong to the Hospital Universitario Clínica San Rafael and must be of legal age and 3) All S-HCWs who have signed the informed consent. According to the eligibility criteria, 62 S-HCWs from the operating room participated in the study.

2.3. Instruments for sampling

Data collection was divided in two parts. On the one hand, a checklist was implemented to collect sociodemographic information from S-HCWs such as genre, age, profession, surgical medical specialty, and professional time. Regarding the S-HW process, the checklist considered all recommendations established by the WHO [21], assessing 1) S-HW direction, 2) surgical soap used and 3) number of times and time of S-HW performed. The section related to number of times of S-HW was focused on evaluating the steps to wash hands, wrists, forearms, and elbows. Additionally, the checklist was designed to assess if S-HCWs properly wear both surgical garments and personal protection items. On the other hand, two forms were designed to collect the bioluminescence data before and after performing the S-HW process. The first form was used to collect ATP levels on S-HCW hands. The organic material of S-HCW hands was collected using an UltraSnap™ Surface ATP Test (Hygiena, USA). The second form was designed to collect the bacteria loading of S-HCW hands; specifically, *EB* and *E. coli*. The swabs to collect bacteria samples were MicroSnap™ *EB* and MicroSnap™ *E. coli* (Hygiena, USA), respectively. Samples were collected in dominant hands of S-HCWs. Swabs were rubbed randomly through all fingers, nails, interdigital areas and entire palm. Sample collection time was approximately 10-20 seconds for each swab. After sample collection, swabs were analyzed with an EnSure

Luminometer (Hygiena, USA), measuring both ATP and bacteria (*EB* and *E. coli*). The luminometer reports results in RLU, which are directly proportional to ATP concentrations, where the higher the RLU number, the more ATP present, and the dirtier the hand. Regarding the bacteria concentrations, RLU values are equivalent to CFU, which were calculated for *EB* and *E. coli* according to the manufacturer instructions (https://www.hygiena.com/food-safety-solutions/indicator-organisms/).

2.4. Collection and analysis of samples

Samples derived from luminometry measurements were taken in the surgical area of the hospital. Swabs were marked with a code that was known only by researchers. Swabs used to measure ATP levels were analyzed once they were taken from S-HCW hands according to manufacturer instructions. Data derived from this first measure were tabulated in a database for later analysis. Swabs to collect bacteria samples were rubbered at the same time; however, they were stored and transported in a Styrofoam cooler with cooling gel bags to be analyzed in the laboratory in a period no longer than two hours. Bacteria samples were incubated for a period of 6 h at 37 °C according to manufacturer protocols in bacteria incubators (Thermo Scientific - 50125882, USA). Once swabs were incubated, they were analyzed with the luminometer. Data derived from this second measurement were tabulated in a database for later analysis. Once swabs were analyzed, they were discarded considering the established inactivation and disposal protocols.

2.5. Bias and error control

S-HCWs were notified in advance about the process to be carried out to reduce the possibility of bias and error. Once S-HCWs confirmed their participation, they were informed that they would not be notified about the date and time in which bioluminescence assays would be taken. Samples were taken in both the morning and afternoon of working days. Special codes were randomly generated through a database to identify S-HCWs. Sampling was conducted by three researchers, who carried out the respective analysis. Data was reviewed by two researchers who verified the quality of the data. Sampling was collected before and after HCWs performed S-HW; additionally, all health personnel were asked not to dry their hands with any paper to avoid any cross contamination.

2.6. Statistical analyses

ATP, *EB* and *E. coli* were statistically compared with variables such as medical specialty, S-HW direction, type of surgical soap, number of times S-HW was performed and amount of time S-HW was performed. Normality analyses of the variables were performed using either Shapiro-Wilk or Kolmogorov-Smirnov tests, depending on the amount of data to be analyzed. Given that the data did not approve the assumption of normality, these were analyzed through non-parametric statistics using the U Mann-Whitney test. The Chi-square (χ^2) test was performed to analyze the association between the reduction of ATP levels with the absence or presence of *EB* and *E. coli* after S-HW. A risk test was used to obtain the odds ratio (OR) and confidence interval (CI). All analyses were performed using SPSS software (IBM SPSS Statistics V.25).

3. RESULTS

The sociodemographic characterization is shown in **Table 1**. This study was performed to S-HCWs from different medical specialties such as orthopedics (6.45%), otorhinolaryngology (6.45%), anesthesiology (4.84%), plastic surgery (3.23%) and urology (3.23); additionally, health personnel such as general physicians (22.58%), medical residents (11.29) and surgical instrument professionals (41.94%) participated in the study. Here, both female (54.84%) and male (45.16%) were included in the study, where 56.45% of them had been working in their current position for less than 5 years, 19.35% had been working between 6 and 10 years, 16.13% had been performing their professional activities between 10 and 20 years, and 8.06% of them have been working in the hospital for more than 20 years.

Table 1. Sociodemographic characterization of S-HCWs from the hospital.

		Number of participants (n)	Percentage (%)
Gender	Female	34	54.84
	Male	28	45.16
Profession	Physician	36	58.06
	Surgical instrument professionals	26	41.94

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		Number of participants (n)	Percentage (%)
Medical specialty	Surgical instrument	26	41.94
	General surgery	14	22.58
	Residency	7	11.29
	Orthopedic surgery	4	6.45
	Otorhinolaryngology	4	6.45
	Anesthesiology	3	4.84
	Plastic surgery	2	3.23
	Urology	2	3.23
Years of work on current position	≤ 5	35	56.45
	≥ 6 - ≤ 10	12	19.35
	≥ 11 - ≤ 20	10	16.13
	≥ 21	5	8.06

Source: Own work

3.1. Adherence to the S-HW protocol by HCWs

Results derived from the application of the checklist are shown in **Table 2**. Before starting the S-HW procedure, 58.06% of S-HCWs wear all the personal protection elements such as glasses, cap and face mask, while 41.94% of health personnel did not wear them. Here, it was possible to observe that there was no established pattern regarding S-HW direction, due to 46.77% of S-HCWs performing a unidirectional S-HW, while 53.23% carrying out a bidirectional HH. Most of the S-HCWs tend to use chlorhexidine (85.48%) compared with those that use povidone iodine (14.52%). According to the WHO, S-HW should be performed three times; here, 75.81% of S-HCWs accomplished this recommendation, while 12.90% of them performed S-HW twice and 11.29% of health personnel only once. Regarding the time spent washing their hands, most of them spent 5 minutes (88.71%), while 11.29% spent 3 minutes. One of the most important steps during S-HW is smearing the nails; here, it was possible to observe that 56.45% of S-HCWs did not accomplish this procedure, while the rest of them did it once (27.42%), twice (14.52%) and three times (1.61%). Finally, the checklist helped identify the pattern performed during S-HW, where 32.26% of S-HCWs wash their hands, forearms, and elbows in one wash, 29.03% of S-HCWs do not operate a pattern, 27.42% of them first wash the elbow, wash the forearm second and wash the wrist last; finally, 11.29% of health personnel first wash the hand and the forearm and then wash the elbow.

Table 2. S-HW characterization performed by S-HCWs included in the study.

		Number of participants (n)	Percentage (%)
Use of personal protection elements	Yes	36	58.06
	No	26	41.94
S-HW direction	Unidirectional	29	46.77
	Bidirectional	33	53.23
Type of surgical soap	Chlorhexidine	53	85.48
	Povidone iodine	9	14.52
Number of times S-HW implemented	1	7	11.29
	2	8	12.90
	3	47	75.81
Time of S-HW performed	3 min	7	11.29
	5 min	55	88.71
Nail smear performed	0	35	56.45
	1	17	27.42
	2	9	14.52
	3	1	1.61
Pattern performed during S-HW	A	17	27.42
	B	7	11.29
	C	0	0
	D	20	32.26
	E	18	29.03

A. First wash the elbow, second wash the forearm and third wash the wrist.
B. First wash the hand and forearm and second wash the elbow.
C. Wash hands, forearm, and elbow in one wash.
D. Wash everything three times.
E. Does not operate a pattern.

Source: Own work

3.2. Luminometric measurements on S-HCW hands

Luminometric levels of ATP in RLU and *EB* and *E. coli* in CFU on S-HCW hands before and after the S-HW process are shown in **Table 3**. Results evidenced that HCWs had an average of 510.9 RLU before performing S-HW, which is directly proportional to ATP levels. This value indicates that S-HCW hands are extremely dirty; however, ATP levels after S-HW were 54.6 RLU, evidencing a statistically significant decrease ($p = 0.001$). This final value indicates that S-HCW hands are clean according to the swabs manufacturer datasheet. It is noteworthy to mention that S-HCW hands must have an RLU value of less than 60 before entering operating rooms. Here, an additional HW was advised if ATP levels were higher than 60 RLU after having performed the S-HW. Regarding the bacteria levels on S-HCW hands, Table 3 shows the CFU for *EB* and *E.*

coli, indicating that health personnel had 26.6 CFU for *EB* before performing S-HW and 21.8 CFU after completing S-HW. Similarly, S-HCW hands evidenced 4 CFU for *E. coli* before performing S-HW and 3.5 CFU after completing S-HW. Statistical analyses did not evidence a decrease for *EB* ($p = 0.283$) and *E. coli* ($p = 0.679$).

Table 3. Luminometric measurements of ATP, *EB* and *E. coli* on S-HCW hands before (B-H) and after (A-H) S-HW. Statistically significant differences were at $p \leq 0.05$ **.

Bacteria		Value	*p*
ATP (RLU)	B-H	510.9	*0.001***
	A-H	54.6	
***EB* (CFU)**	B-H	26.6	*0.283*
	A-H	21.8	
***E. coli* (CFU)**	B-H	4	*0.679*
	A-H	3.5	

Source: Own work

The relation between the reduction of ATP with the absence or presence of *EB* and *E. coli* after S-HW are shown in **Table 4**. Here, the reduction of ATP on S-HCW hands was not related to absence and presence of *EB* and *E. coli*, indicating that although there was a decrease in hand contamination levels after S-HW, this reduction does not mean lower levels of CFU for *EB* ($p = 0.082$) and *E. coli* ($p = 0.680$).

Table 4. Relation between the reduction of ATP with the absence or presence of *EB* and *E. coli* after S-HW. Statistically significant differences were at $p \leq 0.05$ **.

Bacteria	ATP reduction	Absence (%)	Presence (%)	*p*	OR	CI
EB	**YES**	23 (37.1)	23 (37.1)	*0.082*	0.33	0.094 – 1.181
	NO	12 (19.4)	4 (6.5)			
E. coli	**YES**	32 (51.6)	14 (22.6)	*0.680*	0.76	0.209 – 2.77
	NO	12 (19.4)	4 (6.5)			

Source: Own work

Results derived from the reduction or increase of ATP levels and permanence, increase or decrease of *EB* and *E. coli* on HCWs after performing S-HW are shown in **Figure 1**. On the one hand, it was possible to observe either a reduction or increase of ATP in all S-HCWs, where 74.19% of them experienced a decrease in ATP levels after S-HW, while 25.81% did not experience a reduction. On the other hand, a small population (6.45%) evidenced an increase in ATP after S-HW, indicating that 93.55% of all S-HCWs did not reveal an increase in ATP (**Figure 1A**). On the other hand, there were 33.87% and 61.19% of S-HCWs who did not have *EB* (**Figure 1B**) and *E. coli* (**Figure 1C**), respectively. The permanence of *EB* (19.35%) and *E. coli* (20.97%) on S-HCW hands after completing S-HW is shown in Figure 1C. Additionally, there was a population of 19.35% and 6.45% S-HCWs who experienced an increase of *EB* and *E. coli* after performing S-HW, respectively (**Figure 1C**). Finally, there were S-HCWs who demonstrated a decrease in both *EB* (27.42%) and *E. coli* (11.29%) after practicing S-HW (**Figure 1C**).

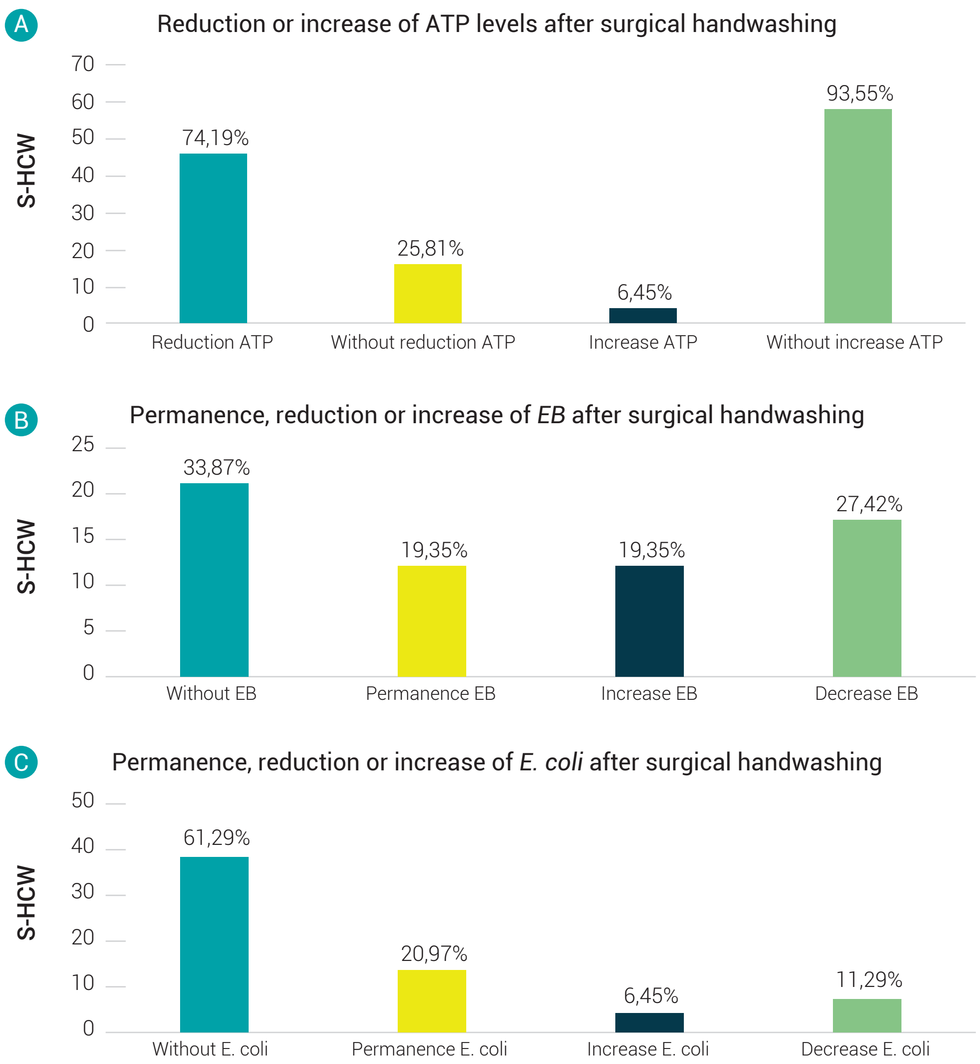

Figure 1. Illustrative bar graphs on the permanence, increase and decrease of **A)** ATP, **B)** *EB* and **C)** *E. coli* in S-HCWs after S-HW.

Source: Own work

A comparison was performed to identify ATP, *EB* and *E. coli* levels between different medical specialties that participated in the study (**Table 5**). Results evidenced that ATP levels were reduced significatively after S-HW for surgical instrument professionals (*p=0.001*), general surgeons (*p=0.001*), residents (*p=0.017*), orthopedists (*p=0.029*) and otolaryngologists (*p=0.029*). No significant differences in the reduction of *EB* and *E. coli* were found. However, in some cases it was possible to observe that levels of *EB* and *E. coli* increased after performing S-HW. Given that anesthesiologists, plastic surgeons and urologists were a small population, it was not possible to statistically analyze the data.

Table 5. Comparison of ATP, *EB* and *E. coli* levels between different medical specialties before (B-H) and after (A-H) S-HW process. Statistically significant differences were at $p \leq 0.05$ **.

Medical specialty	ATP level (RLU)		*p*	*EB* (CFU)		*p*	*E. coli* (CFU)		*p*
	B-H	A-H		B-H	A-H		B-H	A-H	
Surgical instrument professionals	422.9	69.0	*0.001***	32.7	21.2	*0.098*	3.8	2.3	*0.333*
General surgery	488.5	35.9	*0.001***	25	21.4	*0.710*	4.3	2.9	*0.450*
Residency	599.9	71.7	*0.017***	21.4	21.4	*1*	5.7	5.7	*1*
Orthopedic surgery	1136.3	42.3	*0.029***	37.5	12.5	*0.186*	5.0	7.5	*0.617*
Otorhinolaryngology	490.5	36.5	*0.029***	12.5	25.0	*0.495*	2.5	5.0	*0.495*
Anesthesiology	357.7	60.3	---	33.3	33.3	---	3.3	3.3	---
Plastic surgery	233.0	27.5	---	0.0	25.0	---	0.0	5.0	---
Urology	798.0	16.5	---	0.0	25.0	---	5.0	5.0	---

Source: Own work

Another comparison was implemented to identify which factors have a direct incidence in the reduction of ATP, *EB* and *E. coli* (**Table 6**). Here, S-HW direction reduced ATP levels regardless of whether it is unidirectional (*p=0.001*) or bidirectional (*p=0.001*). Regarding the surgical soap used, chlorhexidine efficiently reduced ATP levels (*p=0.001*), while povidone iodine did not have a significant decontaminating effect (*p=0.200*). Here, it was possible to confirm that the number of times S-HW is performed is relevant to the reduction in ATP levels, elucidating that once is not enough to reduce ATP (*p=0.180*), while twice (*p=0.005*) or three times (*p=0.001*) are adequate to eliminate the microbial load almost entirely. The amount of time that S-HW is implemented is crucial for the eradication of all organic material, concluding that three (*p=0.004*) or five (*p=0.001*) minutes are enough to reduce ATP levels. Even though in all cases a reduction in the *EB* and *E. coli* levels was observed, no significant differences were found.

Table 6. Comparison of ATP, *EB* and *E. coli* levels between different S-HW processes performed by HCWs. Data were compared before (B-H) and after (A-H) S-HW. Statistically significant differences were at *p ≤ 0.05 **.*

		ATP level (RLU)		*p*	*EB* (CFU)		*p*	*E. coli* (CFU)		*p*
		B-H	A-H		B-H	A-H		B-H	A-H	
S-HW direction	**Unidirectional**	449.9	38.0	*0.001***	27.6	22.4	*0.435*	5.17	4.83	*0.816*
	Bidirectional	564.5	69.1	*0.001***	25.8	21.2	*0.463*	3.03	2.42	*0.732*
Type of surgical soap	**Chlorhexidine**	524.7	37.2	*0.001***	25.5	21.7	*0.439*	4.53	3.96	*0.663*
	Povidone iodine	429.6	156.8	*0.200*	33.3	22.2	*0.357*	1.1	1.1	*1*
Number of times S-HW implemented	**1**	375.6	143.9	*0.180*	28.6	28.6	*1*	1.4	1.4	*1*
	2	756.0	40.37	*0.005***	28.6	12.5	*0.143*	1.1	1.1	*0.333*
	3	339.1	61	*0.001***	7.1	14.3	*0.538*	1.4	5.7	*0.942*
Time of S-HW performed	**3 min**	682.9	42.0	*0.004***	28.57	21.43	*0.606*	2.86	2.86	*1*
	5 min	489.0	56.2	*0.001***	26.4	21.8	*0.342*	4.18	3.64	*0.664*

Source: Own work

4. DISCUSSION AND CONCLUSION

The HH carried out by HCWs is a recurring practice in their work environment, since this act of cleaning prevents the transmission of diseases from health personnel to patients and vice versa [22]. However, the assessment of surgical hand disinfection processes performed by HCW has not been very well studied and analyzed in low-income countries. Therefore, this study focused on evaluating S-HW in health personnel working in operating rooms of a fourth level Colombian hospital. Accordingly, this study identified that 75.81% of HCWs performed the procedure three times, as established by the WHO [21]; nevertheless, health personnel did not follow a specific pattern when washing their hands, wrists, forearms and elbows. The study developed by Gülşen et al., led to the observation that operating room staff did not display appropriate S-HW techniques such as washing their hands and arms up to 3– 5 cm above their elbows with circular motions for the full time [23]; the estimated time for a good S-HW is around 5 minutes. In our study, 88.71% of HCWs spent 5 minutes doing the complete wash, while 11.29% of S-HCWs spent 3 minutes. According to some studies, performing a S-HW for more than 3 minutes before surgery is enough to reduce bacterial counts [24]–[26]. In fact, operating room staff who did not wash in the proper time, improved their practice after respective feedback on good S-HW practice was given [27]. Another important factor to reduce surgical site infections (SSI) is the nail cleaning. In this study, nail smear was accomplished three times by 1.61% of HWCs, followed by 14.52%, 27.42% and 56.45% (Table 2) who performed nail cleaning two times, one time and never during S-HW, respectively. According to Tanner et al., nail cleaning and scrubbing is an important step during S-HW, because it reduces the number of bacteria [28]. In fact, the study performed by Okgün-Alcan et al., demonstrated that a good S-HW which includes nail cleaning reduces the number of CFU, even if picks or brushes are used to clean nails [29].

A comparative study conducted by Lai et al., evidenced that surgical soap is crucial in the elimination of bacteria in physicians who work in standard operating facilities. In their study, they elucidated that S-HCW using Avagard soap (61% ethyl alcohol, 1% chlorhexidine gluconate) obtained a significantly decrease on CFU (0 CFU) compared to those who used povidone iodine (12.5 CFU) [30]. Similarly, in the work conducted by Tsai et al., after surgical hand disinfection, the mean CFU counts of conventional chlorhexidine (0.8 ± 0.8 CFU) were significantly lower than that of conventional povidone iodine group (3.9 ± 0.8 CFU) [31]. Similar results were obtained in this study, where HCWs who used chlorhexidine reported higher CFU before S-HW (*EB* = 25.5 CFU and *E. coli* = 4.53 CFU) and lower CFU after S-HW (*EB* = 21.7 CFU and *E. coli* = 3.96 CFU). On the contrary, HCWs who used povidone iodine reported higher CFU before S-HW (*EB* = 33.3 CFU and *E. coli* = 1.1 CFU) and lower CFU after S-HW (*EB* = 22.2 CFU and *E. coli* = 1.1 CFU). Although there was a reduction in *EB* and *E. coli*, this decrease was not statistically significant. According to previous results, it was possible to observe that povidone iodine has less effect on reducing bacteria and organic material on S-HCW hands. Even though it has been demonstrated that povidone iodine diluted to a 1% concentration or lower can be applied safely to wounds [32], this dilution is more likely to be ineffective in local bacterial control [33], resulting in povidone iodine having a shorter protective effect against bacterial buildup on the skin after HW [32]. In this sense, chlorhexidine is a better option than povidone iodine for skin preparation when a substantive effect is desired for procedures such as skin preparation for surgery or HW before surgery [34].

One of the purposes of this work was to assess if S-HW eliminates or reduces bacteria on hands such as *EB*, especially *E. coli*. Here, both *EB* and *E. coli* were reduced after S-HW, but this decrease was not statistically significant. Additionally, it was demonstrated that a reduction of ATP is not directly related to the decrease of bacteria on hands. This finding coincides with results obtained by other studies, where

CFU reduction rates did not correlate with the ATP reduction rate, probably because ATP exists in bacteria as well as in nonbacterial sweat and skin drop debris [17]. On the contrary, a study indicated that there is a positive correlation between microbial counts detected by standard culture and ATP levels measured with a commercial kit [18]. It is relevant to highlight that, in this study, *EB* and *E. coli* had a permanence of 19.35% and 20.97% after performing S-HW, respectively. Moreover, *EB* and *E. coli* increased 19.35% and 6.45% after HW, respectively. This increase can be explained by touching surfaces once the hand HW was over. Although in this study *EB* and *E. coli* carriage were identified, there are other kind of bacteria present on HCW hands. For example, a study assessed bacteria elimination on physician hands after tap water or alcohol wash. A reduction in *S. aureus*, Coagulase-negative *Staphylococci*, *E. coli, Pseudomonas aeruginosa, Enterococci spp.* and *Klebsiella pneumoniae* was observed after HW with simple tap water washing (76%) and alcohol HW (16.5%) [35]. The study conducted by La Fauci et al., identified *Staphylococcus spp.* (5.92%), *EB* (3.95%), *Pseudomonas spp.* (1.97%) and *Acinetobacter spp.* (1.97%) [36]. The work carried out by Chrinius et al., identified *S. aureus* in all nurses' hand swabs, concluding that this result is derived of poor HH after using the toilet [37]. Another study found that hands of consultants, residents, internees and nurses are prone to becoming contaminated with *Micrococci spp.* (27.38%), *Bacillus subtilis* (7.6%), Methicillin-Sensitive *S. aureus* (3.82), *Pseudomonas spp.* (2.54%), MRSA (1.27%) and *Acinetobacter spp.* (0.63%). Here, the own work mentioned that adherence to strict HW protocols before and after touching patients will definitely bring down bacteria carriage in HCWs [38]. Health personnel who work in intensive care units (ICU) are exposed to being contaminated with bacteria; for instance, doctors and nurses from a cardiac ICU were colonized by *Acinetobacter spp.* (14.28%), *Bacillus* (9.52%), *Citrobacter* (4.76%), *Klebsiella* (4.76%) and *Serratia* (4.76%). Here, the own work indicated that HW reduces hand colonization with infections; however, it requires HCWs' compliance with optimal practices [39]. The work developed by Eksi et al., not only identified bacteria on HCW hands, but also analyzed the bacteria according to ward location. They evidenced that hands of HCWs who work on pediatrics (newborn), internal medicine (cardiology), coronary ICU, internal medicine ICU, surgical ICU, surgical clinics and adult and pediatric oncology were colonized by *Acinetobacter spp.*, *Enterococcus spp., S. aureus*, *Pseudomonas spp.*, *Enterobacter aerogenes*, *Serratia marcescens*, *Streptococcus* and *Pneumoniae* [40]. Accordingly, several bacteria can be found in HCW hands, even in those who do not work directly in operating rooms. *S. aureus* is one of the bacteria most found on HCW hands [35], [36], [38], [41], [42]. According to some own work, HCW with *S. aureus* have a greater possibility to spread this infection to the community at large because of their interaction and exposure to the hospital environment, causing major risks in transmission to hospital patients and spreading this type of nosocomial infection [43], [44]. On the other hand, *E. coli* has been isolated in a lower proportion on HCW hands [35], [45], [46]; however, it is important to highlight that *E. coli* is listed as one of the most important pathogenic bacteria causing nosocomial infections. In fact, the increasing antibiotic multidrug resistance of this bacteria has created many problems [47]. It has been described that coliforms are fecal bacteria and their presence suggests that food and water supplies could be the source of contamination [48].

The analysis performed here not only provided insight into how HCWs are implementing the S-HW protocol, but also identified which factors need to be considered, assuring a correct elimination of both organic material and bacteria. However, this study has some limitations; for instance, the size of population analyzed was lower; therefore, it is necessary to include more S-HCWs from different hospitals in the region. In this way, it will be possible to achieve a more robust characterization of the main risk factors that can trigger HAIs from poor HH. On the other hand, it was not possible to analyze other kinds of bacteria such as *S. aureus*, *Pseudomonas aeruginosa*, *Klebsiella,* among others. The main reason is that the kit identifies mesophylls in general and no other specific types of microorganisms. It is necessary to implement other protocols such as glove juice, which has been widely used to collect hand samples, followed by *in vitro* cultures [49]; in fact, the glove juice method is a simple, easy and practical technique for the determination of colonization of HCW hands and can be adapted as a methodology for screening HH [38]. Another limitation of this work lies in the lack of identification of resistance patterns to antimicrobial agents. This is relevant to form a picture of the degree of resistance to each drug in the population at large [50]. For example, some studies have assessed the resistance percentage of *EB*, showing a resistance to ceftriaxone (41%), cefoxitin (44%), mecillinam (52%) and imipenem (33%) [36]. Similarly, the antibiotic susceptibility profile for *E. coli* has evidenced that this bacteria is resistant to ampicillin (7.7%) and tetracycline (46.2%) [37], ceftriaxone (100%), ciprofloxacin (100%), gentamycin (100%), ceftazidime (100%) and cefotaxime (100%) [44]. Finally, it would have been useful to perform an analysis of hands after surgical interventions to observe if HCWs keep or increase the organic material and bacteria in their hands [31]. According to these limitations, we consider our preliminary results to be the starting point for future studies, including a well-designed, more reliable, objective, and quantitative study that accurately estimates S-HW technique HCWs in Colombia.

Overall, this study elucidated that S-HW effectively decreased ATP levels; however, this HH technique did not evidence a significant reduction on bacteria, specifically, *EB* and *E. coli*. Although there was a reduction in organic material, it is crucial to mention that this reduction was evidenced with chlorhexidine, but not with povidone iodine. On the other hand, it was possible to observe that total time and number of times that S-HW are performed are decisive in reducing ATP, concluding that more than twice for 3 minutes or more are necessary to significantly reduce hand contamination. Having analyzed the data, it cannot be concluded that HCWs could exclusively be transmitters of bacteria at the hospital level, specifically in operating rooms. Nevertheless, medical training is an indicative to promote adequate HH behaviors in daily practice, as well as periodic screening to detect bacteria with pathogenic potential and to be able to carry out an intervention that limits the circulation of HAIs. Likewise, it will be of future interest to relate the microbiological findings with the incidence of HAIs with these microorganisms during the rotation time of HCWs in each service.

ACKNOWLEDGMENT

The own work gratefully thank the Teaching and Research Department from the Hospital Universitario Clínica San Rafael for supporting us in the realization of this project. Additionally, we are grateful to all the physicians and surgical instrument professionals who participated in the study.

FUNDING

This study was supported by the Dirección Nacional de Investigación from the Fundación Universitaria del Área Andina through grant No. CV2021-CSD-NBT-B-12285.

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