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ORIGINAL ARTICLE

Determination of the Effects of Hand Hygiene Education Given to Nursing Students in Intensive Care Unit on Hand Microbiota

Yoğun Bakım Ünitesinde Hemşirelik Öğrencilerine Verilen El Hijyeni Eğitiminin El Mikrobiyotasına Etkisinin Belirlenmesi

[1]Fadime Özdemir, [2]Hülya Saray Kılıç, [2]Buse Sungur, [2]Esra Aydın, [2]Merve Taşcı

[1]Department of Molecular Biology and Genetics, Faculty of Science, Bilecik Şeyh Edebali University
[2]Faculty of Health Sciences, Department of Nursing, Bilecik Şeyh Edebali University

Correspondence

Fadime Özdemir, Department of Molecular Biology and Genetics, Faculty of Science, Bilecik Şeyh Edebali University

E-Mail: fadime.ozdemirkocak@bilecik.edu.tr

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ABSTRACT

Purpose: This study is conducted with the purpose of comparing fourth-year nursing students' hand hygiene practices and beliefs with their hand flora. Additionally, we aim to assess the effectiveness of hand hygiene education in this context.
Material and Methods: This research has been conducted using a pre-test, post-test, and control group quasi-experimental design. The research was conducted with fourth-grade students studying at the Nursing Department of a Faculty of Health Sciences in the fall semester of the 2022-2023 academic year. Twenty students who volunteered to participate were included using a simple random sampling method and were then randomly assigned to experimental and control groups. The Hand Hygiene Application Inventory and the Hand Hygiene Belief Scale administered as pre-tests. Students underwent tape stripping and swab methods to collect samples for their hand flora before any interventions. Ten students in the experimental group were given education about hand hygiene rules by the researchers. A post-test was conducted for the experimental group.
Results: The hand hygiene beliefs and practices of both groups were similar before training. When the Hand Hygiene Application Inventory and Hand Hygiene Belief Scale scores before and after the training were examined, an increase was observed in the scale scores after training. In this study, the microorganisms present in the general hand flora were identified simplistically, and the changes were observed after hand hygiene education. Furthermore, the study shed light on the structure of hand flora after education in terms of microbial load.
Conclusion: It has been experimentally determined that there is a significant decrease in hand microbiota load and pathogenic groups with hand hygiene education.

Keywords: Hand hygiene, Hand microbiota, Intensive care, Nursing student.

ÖZ

Amaç: Bu çalışma hemşirelik dördüncü sınıf öğrencilerinin el hijyeni uygulama ve inançlarının el florası ile karşılaştırılması amacıyla yapılmıştır. Ayrıca bu bağlamda el hijyeni eğitiminin etkinliğini değerlendirmeyi amaçlıyoruz.
Gereç ve Yöntemler: Bu araştırma ön test, son test ve kontrol gruplu yarı deneysel desen kullanılarak gerçekleştirilmiştir. Araştırma, 2022-2023 akademik yılı güz döneminde bir Sağlık Bilimleri Fakültesi'nin Hemşirelik Bölümü'nde öğrenim gören dördüncü sınıf öğrencileri ile gerçekleştirilmiştir. Araştırmaya katılmaya gönüllü olan yirmi öğrenci basit rastgele örnekleme yöntemi kullanılarak seçilmiş ve daha sonra deney ve kontrol gruplarına rastgele atanmıştır. El Hijyeni Uygulama Envanteri ve El Hijyeni İnanç Ölçeği ön test olarak uygulanmıştır. Öğrencilere herhangi bir müdahale öncesinde el florası için örnek toplamak amacıyla bant çıkarma ve sürüntü alma yöntemleri uygulanmıştır. Deney grubundaki 10 öğrenciye araştırmacılar tarafından el hijyeni kuralları hakkında eğitim verilmiştir. Deney grubuna son test uygulanmıştır.
Bulgular: Her iki grubun el hijyeni inançları ve uygulamaları eğitim öncesinde benzerdi. El Hijyeni Uygulama Envanteri ve El Hijyeni İnanç Ölçeği puanları eğitim öncesi ve sonrası incelendiğinde eğitim sonrası ölçek puanlarında artış gözlendi. Bu çalışmada genel el florasında bulunan mikroorganizmalar basit bir şekilde tanımlanmış ve el hijyeni eğitimi sonrası değişimler gözlemlenmiştir. Ayrıca çalışma, mikrobiyal yük açısından eğitim sonrası el florasının yapısına ışık tutmaktadır.
Sonuç: El hijyeni eğitimi ile el mikrobiyota yükünde ve patojen gruplarda anlamlı bir azalma olduğu deneysel olarak belirlenmiştir.

Anahtar kelimeler: El hijyeni; el mikrobiyotası; yoğun bakım; hemşirelik öğrencisi.

Introduction

Healthcare-associated infections in intensive care units are important causes of preventable morbidity, mortality, and prolonged length of stay (1). The management of infectious diseases is progressively becoming more difficult, primarily due to the emergence of resistance mechanisms in bacteria. Non-adherence to proper hand hygiene stands as a paramount factor among the leading causes of healthcare-associated infections. Emphasizing proper hand hygiene practices in intensive care units is highlighted as a crucial measure for managing central catheter-associated infections (2) and controlling ventilator-associated pneumonia (3). In a recent study, it was observed that healthcare professionals exhibit a higher frequency of transitioning from tasks involving contamination to those involving cleanliness during patient care responsibilities. This dynamic has the potential to elevate the risks of transmission and infection (4). Due to the higher likelihood of healthcare workers transmitting pathogenic microorganisms to intensive care patients through their hands, addressing the knowledge and implementation gaps in hand

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hygiene practices among the staff is emphasized as an effective strategy in reducing infection rates (5).

In a study evaluating the hand hygiene practices of nurses in the hospital, it was found that only 65% of participants demonstrated proper hand hygiene compliance (6). To reduce the spread of healthcare-associated infections, continuous education is recommended for intensive care unit staff to induce behavioral changes in hand washing practices (3). A study focused on the adherence of neonatal intensive care unit nurses to standard infection control measures and the identification of facilitating factors revealed that clinical experience and educational qualifications are fundamental determinants in this regard (7). The study recommended periodic updates and evidence-based training related to the subject, highlighting the significance of staying current in the field (7). It has been observed that compliance with hand hygiene can be enhanced up to six months following the conducted training interventions (8). Therefore, it is recommended that the training programs are of high quality, tailored to the specific goals, and inclusive of all intensive care staff members (9).

Since nursing students are considered healthcare workers during their education, they could potentially serve as vectors for cross-contamination in the hospital setting (10). Additionally, due to the elevated risk of exposure to occupational biological hazards, it is recommended to provide training for students to enhance their knowledge and compliance with infection prevention practices (11). Training interventions have been found to foster positive attitudes towards hand hygiene and enhance awareness (10-12). While studies have determined that nursing students hold positive beliefs and value hand hygiene (13), there is also recognition of the need to improve compliance with hand hygiene (14, 15). The use of hand hygiene training videos has been observed to increase nursing students' handwashing skills and compliance rates (12). However, it is important to note that the outcomes of these studies are based on students' self-reports. Therefore, there is a need for observation-based studies concerning compliance with hand hygiene (12,13-16,17). As a result, this approach can enable healthcare workers to provide care to patients in a more efficient, effective, and safe manner (4).

The skin, our largest organ, possesses two distinct microbiotas: one permanent and one temporary, both enveloping the body. Human skin provides a habitat for commensal microbiota while also serving as a physical barrier to deter the invasion of foreign pathogens. It is colonized by various microbiotas, playing a dual role of hosting and repelling potential intruders (18).

The microbial load on hands varies considerably in comparison to other skin areas, with healthcare workers' hand microbial load reported to be between 3.9x104 and 4.6x106 CFU/cm2 (19). Different isolation methods can be employed to determine the presence of microorganisms in an environment (20, 21). Sampling through the swab method from clinical specimens is a widely used approach (22). In a study, the tape stripping method was compared to the swab method for collecting live skin bacteria without compromising the composition of the skin microbiome. It was demonstrated that the tape stripping method is comparable to the swab method (23).

The literature does not currently include a study comparing nursing students' hand hygiene practices and beliefs with their hand flora. Therefore, our research is conducted with the purpose of comparing fourth-grade nursing students' hand hygiene practices and beliefs with their hand flora. Additionally, we aim to assess the effectiveness of hand hygiene education in this context.

The hypotheses of the study are as follows:

H0: Hand hygiene training has no effect on hand microbiota load and pathogenic group amount.

H1: Hand hygiene training reduces hand microbiota load and pathogenic groups.

Material and Methods

Type of research

This research has been conducted using a pre-test, post-test, and control group quasi-experimental design.

Research population and sample

The research was conducted with fourth-grade students studying in the Nursing Department of a Faculty of Health Sciences in the fall semester of the 2022-2023 academic year. For the specified academic year, 20 volunteer students practicing in intensive care unit within the scope of clinical practice course were randomly selected by the instructor in charge of the course. Students were assigned to the experimental and control groups by using the lottery method.

Implementation of the research

The data of the study was collected between September and December 2022. Before the intervention, students in both groups were administered the Hand Hygiene Application Inventory (HHAI) and the Hand Hygiene Belief Scale (HHBS) as pre-tests. All students underwent tape stripping and swab methods to collect samples for their hand flora before any interventions. Ten students in the experimental group were given education about hand hygiene rules by the researchers. Ten students in the control group did not participate in the education. After the education, the students continued to care for patients in the intensive care unit. A post-test was conducted for the experimental group after the education. Subsequent to application, all students underwent sample collection using the same methods.

Implementation of hand hygiene education

The content of the hand hygiene education was designed in alignment with the existing literature. It encompassed key aspects such as proper handwashing techniques, the products utilized during

handwashing, strategies for ensuring effective hand hygiene, recommended frequency of handwashing, scenarios necessitating handwashing, potential barriers to handwashing, and the appropriate usage of alcohol-based antiseptics.

The training session took place in the hospital's designated meeting room and was structured to last for approximately 45 minutes. The content was tailored to suit the educational needs of the participants and was aimed at providing comprehensive insights into the topics outlined above.

Data collection instruments

Hand Hygiene Application Inventory (HHAI) and Hand Hygiene Belief Scale (HHBS): The HHAI is a 14-item inventory used to assess hand hygiene practices; whereas the HHBS is a 22-item scale designed to evaluate beliefs regarding hand hygiene. The Turkish validity and reliability of these instruments were established by Karadağ et al. in 2016 (24).

Both scales utilize a 5-point Likert scale. Scores for HHAI range from a minimum of 14 to a maximum of 70 while scores for HHBS range from a minimum of 22 to a maximum of 110. These instruments were employed to assess the participants' hand hygiene practices and beliefs in the context of the study.

Determining hand flora

To ascertain changes in the hand flora of participating students who received hand hygiene education and those who did not, isolation studies were conducted using swab and tape stripping methods on skin flora. This allowed for the revelation of the microbial profile. General and selective culture media were employed in the isolation process. Nutrient agar served as the general culture medium while Blood agar and Brain Heart Infusion (BHI) agar functioned as selective media.

Nutrient agar is a general-purpose medium suitable for the growth of various organism groups. Blood agar is a selective medium employed for the isolation of Streptococcus pneumoniae, other streptococci, Bacteriodes, Clostridium and yeast. BHI agar, being an enriched medium, can be used for the culture of challenging-to-culture organisms like streptococci, pneumococci and meningococci. The utilization of these media aided in determining the microbial composition present in the hand flora of the participants.

Swab Method

Sterile swab sticks were gently rubbed onto the surface of the hand to collect samples. Subsequently, the swab stick was cut and placed into a vial containing Ringer's solution, where it was left to stand for 30 minutes. Serial dilution was performed using the dilution plate method, and 200 µl was taken from each dilution to be inoculated onto prepared culture media using the streak plate method (21).

Tape Stripping Method

Acrylic adhesive medical permeable tape was sterilized using ultraviolet radiation and then applied to the hands of nursing students for a duration of one minute. Subsequently, using sterile forceps, the tape was peeled off the skin and, similar to the swab method, inoculated onto culture media using the dilution plate method. Following this, the plates were left to incubate at 30 °C and 37 °C for 24 hours, and the resulting colony formations were examined for differentiation. All colonies that had formed as single entities were subjected to tests for catalase and coagulase enzyme activities. Based on the test outcomes, the colonies were grouped into fundamental categories.

Determination of Isolated Bacteria's Enzyme Activities

Catalase Test

Catalase activity of microorganisms that appeared as single colonies on isolation plates was determined using hydrogen peroxide. For this test, S. aureus was used as a control group. The catalase test is employed for identifying aerobic bacteria containing cytochrome with catalase enzyme activity, along with certain facultative bacteria. A pure single colony of the microorganism from the solid culture medium was aseptically transferred onto a glass slide. A few drops of 3% hydrogen peroxide (H_2O_2) were added onto the colony using a sterile loop. The formation of bubbles after the reaction was evaluated as a positive result.

Coagulase Test

Coagulase enzyme, which is involved in plasma clotting and also possesses deoxyribonuclease (DNase) characteristics, is widely used for identifying staphylococci. It is particularly employed to determine the pathogenicity of staphylococci. In the coagulase test, S. aureus was used as the control group.

A clean glass slide was prepared, and a pure colony from the agar plate was suspended in sterile water. A few drops of fresh sterile human plasma were added onto the suspension. The clotting status was assessed after 3-5 seconds, and the result was determined as either positive or negative. This test is significant for evaluating the pathogenicity of staphylococci.

Data Analysis

The research data were analyzed using SPSS 21.0 software package. Descriptive statistics were presented in terms of numbers, percentages, means, and standard deviation values. For comparative analyses, the Mann-Whitney U test was used for independent groups, while the dependent t-test was employed for dependent groups. A significance level of $p<0.05$ was considered to determine the statistical significance of the results.

Ethical Considerations

The research was conducted in accordance with the Helsinki Declaration. Prior to implementation, approval was obtained from the Bilecik Şeyh Edebali University Non-Interventional Clinical Research Ethics Committee

with approval number 7/6 and date 22.11.2022. Permission for conducting the study at the intensive care unit where the students practiced was obtained, and written and verbal consent was obtained from the participants. The researchers informed the participants about the research topic, purpose, and process. Ethical principles were strictly followed throughout the study.

Results

The pre-training and post-training results of the HHAI and HHBS applied to the experimental and control groups are presented in Table 1. Before training, the experimental group had a mean HHAI score of 61.40±4.42 and an HHBS score of 79.30±5.18. The control group had a mean HHAI score of 64.30±4.16 and an HHBS score of 79.30±16.14 before training. After training, the experimental group's mean HHAI score was determined as 69.30±0.94, and the HHBS score was 94.60±5.25. There was no statistically significant difference in the HHAI and HHBS scores before training between the experimental and control groups (p>0.05). This indicates that the hand hygiene beliefs and practices of both groups were similar before training. When comparing the pre- and post-training HHAI and HHBS scores within the experimental group, a statistically significant increase was observed in scale scores after training (p<0.05).

Isolation to collect samples from the skin flora of both the experimental and control group students was conducted using the swab and tape stripping methods. Samples obtained from all students using these two methods were inoculated onto three different culture media using the dilution plate method. After the incubation period, bacterial colonies that developed on the plates were examined, and colony counts were performed. The obtained results are presented in Table 2. In this study, the aim was to determine bacterial groups in the hand flora using two different methods and three different media. For the samples obtained through the swab method, based on the results from BHI agar and blood agar media, no significant differences were observed in the growth of bacterial colonies before and after contact for both the experimental and control groups (p>0.05). However, when considering the results from the nutrient agar media, a significant difference was detected in the colony counts before and after contact for both groups (p<0.05). Regarding the samples obtained through the tape stripping method, no significant differences were found in colony counts between the experimental and control groups before contact for blood agar and nutrient agar media (p>0.05). However, a significant difference was observed in colony counts before contact for the BHI agar media (p<0.05). After contact, significant differences in colony counts were observed between the experimental and control groups for blood agar, BHI agar, and nutrient agar media (p<0.05). In all media, the experimental group exhibited significantly lower colony counts compared to the control group (p<0.05).

Table 1. HHAI and HHBS Results for Experimental and Control Groups

		Experimental Group (n=10)		Control Group (n=10)		
		Mean	Standard Deviation	Mean	Standard Deviation	p Z
Pre-education	HHAI	61.40	4.42	64.30	4.16	0.211 -1.252
Post-education	HHAI	69.30	0.94			
p Z		0.005 -2.805				
Pre-education	HHBS	79.30	5.18	79.30	16.14	0.363 -0.911
Post-education	HHBS	94.60	5,25			
p Z		0.005 -2.805				

Table 2. Colony Count Ratios of Samples Obtained Through Swab And Tape Stripping Methods In Different Media

	Swab Method					
	Before contact			After contact		
	Experimental Group	Control Group		Experimental Group	Control Group	
	Mean±SD	Mean±SD	p Z*	Mean±SD	Mean±SD	p Z*
BHI agar	1.20±2.25	202.80±419.69	0.195 -1.195	0.80±1.31	219.30±415.35	0.669 -0.428
Blood agar	1.40±3.09	407.10±509.92	0.123 -1.544	2.80±6.51	109.50±313.26	0.609 -0.512
Nutrient agar	3.50±9.68	599.70±515.49	0.024 -2.253	0.20±0.63	304.30±479.58	0.045 -2.008
	Tape Stripping Method					
	Before contact			After contact		
	Experimental Group	Control Group		Experimental Group	Control Group	
	Mean±SD	Mean±SD	p Z*	Mean±SD	Mean±SD	p Z*
BHI agar	1.40±4.08	424.70±495.45	0.003 -2.962	0.90±1.85	307.70±477.26	0.035 -2.112
Blood agar	1.10±3.47	308.00±477.52	0.090 -1.693	0	399.60±515.88	0.029 -2.179
Nutrient agar	1.00±2.82	299.90±482.42	0.130 -1.514	0.50±1.08	401.10±514.60	0.029 -2.187

*Mann whitney u test

Table 3. Comparison of sample collection methods for before and after contact media

		BHI agar		Blood agar		Nutrient agar	
		Before contact	After contact	Before contact	After contact	Before contact	After contact
Swappping	p	0,244	0,476	0,234	0,859	0,152	0,271
Stripping	Z*	-1,165	-0,713	-1,190	-0,178	-1,433	-1,101

*Mann Whitney u test

Table 4. Enzyme test results of bacteria obtained by swab method

Swab method							
		Coagulase Test					
		Blood agar		BHI agar		Nutrient agar	
			n (%)		n (%)		n (%)
Before contact	Experimental Group	+	1(10)	+	0	+	1(10)
		-	1(10)	-	2(20)	-	0
		*	8(80)	*	8(80)	*	9(90)
		T	10(100)	T	10(100)	T	10(100)
	Control Group	+	1(100)	+	1(100)	+	2(20)
		-	5(50)	-	5(50)	-	6(60)
		*	4(40)	*	4(40)	*	2(20)
		T	10(100)	T	10(100)	T	10(100)
		Blood agar		***BHI agar***		Nutrient agar	
			n (%)		n (%)		n (%)
After contact	Experimental Group	+	1(10)	+	3(30)	+	0
		-	2(20)	-	0	-	0
		*	7(70)	*	7(70)	*	10(100)
		T	10(100)	T	10(100)	T	10(100)
	Control Group	+	1(10)	+	1(10)	+	1(10)
		-	3(30)	-	5(50)	-	5(50)
		*	6(60)	*	4(40)	*	4(40)
		T	10(100)	T	10(100)	T	10(100)
		Catalase Test					
		Blood agar		BHI agar		Nutrient agar	
			n (%)		n (%)		n (%)
Before contact	Experimental Group	+	2 (20)	+	5 (50)	+	3(30
		-	2 (20)	-	1 (10)	-	1(10)
		*	6 (60)	*	4 (40)	*	6(60)
		T	10 (100)	T	10 (100)	T	10(100)
	Control Group	+	6 (60)	+	5 (50)	+	5(50)
		-	1 (10)	-	1 (10)	-	0
		*	3 (30)	*	4 (40)	*	5(50)
		T	10 (100)	T	10 (100)	T	10(100)
		Blood agar		BHI agar		Nutrient agar	
			n (%)		n (%)		n (%)
After contact	Experimental Group	+	2(20)	+	3(30)	+	0
		-	0	-	1(10)	-	0
		*	8(80)	*	6(60)	*	10(100)
		T	10(100)	T	10(100)	T	10(100)
	Control Group	+	4(40)	+	4(40)	+	4(40)
		-	0	-	0	-	0
		*	6(60)	*	6(60)	*	6(60)
		T	10(100)	T	10(100)	T	10(100)

+: Positive; -: Negative; *: Not determined; T: Total

Table 5. Enzyme test results of bacteria obtained by stripping with tape

Stripping Method							
		Coagulase Test					
		Blood agar		BHI agar		Nutrient agar	
			n (%)		n (%)		n (%)
Before contact	Experimental Group	+	0	+	0	+	0
		-	0	-	1(10)	-	0
		*	10(100)	*	9(90)	*	10(100)
		T	10(100)	T	10(100)	T	10(100)
	Control Group	+	2(20)	+	0	+	2(20)
		-	4(40)	-	8(80)	-	5(50)
		*	4(40)	*	2(20)	*	3(30)
		T	10(100)	T	10(100)	T	10(100)
		Blood agar		BHI agar		Nutrient agar	
			n (%)		n (%)		n (%)
After contact	Experimental Group	+	0	+	1(10)	+	0
		-	0	-	0	-	0
		*	10(100)	*	9(90)	*	10(100)
		T	10(100)	T	10(100)	T	10(100)
	Control Group	+	1(10)	+	1(10)	+	1(10)
		-	2(20)	-	4(40)	-	6(60)
		*	7(70)	*	5(50)	*	3(30)
		T	10(100)	T	10(100)	T	10(100)
		Catalase Test					
		Blood agar		BHI agar		Nutrient agar	
			n (%)		n (%)		n (%)
Before contact	Experimental Group	+	1(10)	+	1(10)	+	2(20)
		-	0	-	0	-	0
		*	9(90)	*	9(90)	*	8(80)
		T	10(100)	T	10(100)	T	10(100)
	Control Group	+	3(30)	+	6(60)	+	3(30)
		-	0	-	0	-	1(10)
		*	7(70)	*	4(40)	*	6(60)
		T	10(100)	T	10(100)	T	10(100)
		Blood agar		BHI agar		Nutrient agar	
			n (%)		n (%)		n (%)
After contact	Experimental Group	+	1(10)	+	0	+	2(20)
		-	0	-	0	-	0
		*	9(90)	*	10(100)	*	8(80)
		T	10(100)	T	10(100)	T	10(100)
	Control Group	+	1(10)	+	3(30)	+	2(20)
		-	1(10)	-	1(10)	-	2(20)
		*	8(80)	*	6(60)	*	6(60)
		T	10(100)	T	10(100)	T	10(100)

+: Positive; -: Negative; *: Not determined; T: Total

The results of the comparison of colony counts based on sample collection methods and media are presented in Table 3. When the results obtained from the tape stripping and swab methods were compared for both before and after contact conditions, it was determined that there was no statistically significant difference in colony counts between the sample collection methods ($p>0.05$). This suggests that the choice of sample collection method did not lead to significantly different colony counts in terms of culture media, regardless of whether the samples were collected before or after contact.

Isolation plates were used to perform catalase and coagulase enzyme tests on microorganisms that fell as single colonies. S. aureus was utilized as the control group for these tests. Isolates with catalase enzyme activity were considered positive if bubble formation occurred after the reaction. In the coagulase enzyme test, results were evaluated as positive or negative based on whether clotting occurred. The enzyme test results for bacteria obtained through tape stripping and swabbing methods in our study are shown in Table 4 and Table 5. For samples collected using both methods, the proportion of positive organisms in the coagulase test ranged from 10% to 80%. Prior to and after contact, the positivity rate for the experimental group ranged from 0% to 30%, whereas for the control group, it was 10% to 80%. Similarly, in the catalase test, the total positivity rate for the experimental group ranged from 10% to 60%. For the same group, this rate

was 0% to 50% for the control group, both before and after contact. It was observed that the experimental group receiving the education had lower rates of microorganisms with enzyme activity.

Discussion

Handwashing is the simplest, most effective, cost-efficient, and universally applicable method in the prevention of healthcare-associated infections (25). In addition to healthcare workers, nursing students are also among the groups that need to adhere to infection control measures in intensive care units and other hospital units. The purpose of education applied to these care providers is to increase their knowledge, enhance their skills and attitudes during healthcare delivery (5). In our study, nursing students' hand hygiene practices and beliefs were compared with their hand flora, and the effectiveness of hand hygiene education was evaluated.

In our study, the average HHAI scores before education were determined as 61.40±4.42 for the experimental group and 64.30±4.16 for the control group. In other studies, the average HHAI scores were reported as 64.67±5.03 by Türeyen and Artan (26), 63.97±6.37 by Okuroğlu et al. (12), 65.90±5.54 by Alcan and Dolgun (27), 67.42±4.98 by Artuvan and Çetin (28), 65.26±5.29 by Gürlek Kısacık et al. (29), 64.26±5.33 by Bayram et al. (30), 67.2±3.9 by Çakırlı Kozik et al. (31), and 64.96±9.09 by Şahbaz and Adana (32). In our study, the average HHBS scores before education were found as 79.30±5.18 for the experimental group and 79.30±16.14 for the control group. In other studies, the average HHBS scores were reported as 87.50±9.35 by Türeyen and Artan (26), 84.03±8.28 by Okuroğlu et al. (12), 85.04±8.20 by Alcan and Dolgun (27), 87.34±9.73 by Artuvan and Çetin (28), 86.01±9.08 by Gürlek Kısacık et al. (29), 76.00±18.76 by Bayram et al. (30), 82.7±8.7 by Çakırlı Kozik et al. (31), and 87.29±13.34 by Şahbaz and Adana (32).

It can be observed that the mentioned studies were conducted with different healthcare professionals, in various work settings, and some involved students as participants. Our HHAI and HHBS results are in line with the outcomes of these other studies, and overall, it's notable that participants' hand hygiene practices and beliefs tend to be high or close to high in other studies as well. Considering the significant increase in these results after the education provided in our study, the effectiveness of the education cannot be ignored.

In our study, the HHAI and HHBS results of the experimental and control groups were similar before the education. This indicates that the groups were comparable before the intervention. The statistically significant increase in post-test averages compared to pre-test averages after the education indicates that the provided education positively influenced the hand hygiene practices and beliefs of nursing students. In a study by Cruz and Bashtawi (10), it was determined that nursing students had a moderate level of hand hygiene knowledge. In another study, it was determined that providing hand hygiene education through a booklet and historical hand hygiene application significantly improved attitudes in the experimental group compared to the control group (33). In an examination of the effect of practical hand hygiene education provided to auxiliary service personnel on hand hygiene compliance, it was reported that the compliance of auxiliary service personnel working in the operating room significantly increased (34). In another study, it has been noted that hand hygiene education for patient relatives and healthcare workers increased awareness, but it is emphasized that these educations should be repeated to keep the topic on the agenda (35). In studies evaluating educational interventions, positive outcomes from the education are expected. In our study, besides the increase in measurement results after the education, the evaluation of hand flora is considered as a factor that enhances the quality of our study. However, it is recommended that similar trainings should not only target nursing students but should be periodically repeated for all healthcare workers to maintain their effectiveness.

Isolating different microorganisms that can grow vegetatively in various environments is possible through isolation methods (20, 21). Molecular methods such as metagenomic analysis are used to determine the presence of non-culturable bacteria (36). Sampling from clinical specimens or surface environments using the swabbing method is widely used (22). In this study, the differences between the swabbing method and the tape-stripping method were investigated. Additionally, the effectiveness of different media in bacterial isolation was determined. In samples collected using the swabbing method, significant differences in colony counts before and after contact were observed for the nutrient agar medium compared to the BHI agar and blood agar media. Accordingly, it can be inferred that the bacterial growth in the swabbing method and the nutrient agar medium was lower in the experimental group before and after contact, compared to the control group.

In the tape-stripping method, differences were observed in the samples collected before and after contact. Before contact, the tape-stripping method showed significant differences between the experimental and control groups in terms of colony counts on the BHI agar medium. After contact, significant differences in colony counts were observed in all three media. In all media, there was a statistically significant lower level of bacterial growth in the experimental group compared to the control group. As a result of the provided training, we can observe a reduction in microbial load in the samples collected from the experimental group after contact.

Swap and tape string methods are simple but effective invasive methods used for skin isolation. In different studies on these techniques, the effectiveness of the techniques and their advantages have been compared. In a study on the importance of the structure of the swab material used in the swab technique, 15 different swabs consisting of cotton (5), flocked foam

(7) and nylon (3) were used (37). The swab sampling efficiency of these swabs was evaluated and it was stated that cotton swabs and small foam swabs were advantageous in sampling non-absorbent surfaces. It is thought that the cotton swabs used in our study provide the desired level of effectiveness because they are the most suitable material for sampling. In a study conducted with the tape stripping technique, it was determined that the structure of the skin was effective in taking samples (38). In a different study, the washing process was compared with the band stripping method in the detection of antimicrobial peptides, and similar results were obtained in both methods (39). In the study conducted by Ogai et al. (40), swabbing and stripping techniques were compared with molecular methods. When next-generation sequencing results were compared to both sampling methods, it was determined that the tape scraping method collected a higher number and wider variety of live skin bacteria than the swabbing method. When our results were evaluated, it was determined that the tape string method generally yielded more bacteria. However, it has been determined that the medium used also changes the effectiveness of the method. For this reason, carrying out isolation studies with the appropriate method and appropriate medium will ensure more effective results.

In a study conducted by Öz et al. (22), they examined the bacterial composition of hand flora among healthcare workers, medical students, and patients along with their accompanying relatives. Similar to our study, they used biochemical tests to analyze the bacterial structure of hand flora. The results of their investigation revealed the presence of coagulase-negative staphylococci, micrococci, viridans streptococci, and coryneform bacteria. Furthermore, they highlighted that the proportion of bacteria not typically found in normal skin flora was notably high. It is particularly striking that this rate is higher among students. In a study conducted by Yayla using swab samples taken from healthcare workers in the intensive care unit and from inanimate surfaces within the unit, blood agar and Eosin-Methylene Blue (EMB) agar were employed for isolation purposes (41). The results showed growth in 15 samples, which were determined to contain Coagulase-Negative Staphylococcus, S. aureus, E. coli, Proteus spp., and Acinetobacter spp. This study provides insights into the microbial presence in the intensive care unit environment, shedding light on the types of bacteria that can thrive on surfaces and among healthcare workers in that setting.

In a study conducted by Mbanga et al. (42), samples were collected from inanimate surfaces and medical equipment (58 swab samples) as well as from hand swabs of healthcare personnel (six swab samples) within the intensive care unit. The results revealed the presence of various bacteria, including E. coli, Klebsiella spp., S. aureus, Coagulase-Negative Staphylococcus, and P. aeruginosa, in the samples obtained from healthcare personnel. This study provides valuable insights into the microbial composition of both surfaces and healthcare workers' hands within the intensive care unit setting. A study conducted with medical and nursing students in Jordan by Bataineh et al. (43) collected swab samples from hands, stethoscopes, and mobile phones. The results revealed that the highest level of contamination was observed on hands. Since it was determined that hands were the most contaminated surface among the sampled items, this study highlights the importance of hand hygiene practices among healthcare students and professionals.

In this study, it was observed that the proportion of bacteria with coagulase activity in the microorganisms obtained through the swabbing and tape-stripping methods in the experimental group was significantly lower compared to the control group. The coagulase test is commonly used for the identification of staphylococci. It is a test particularly utilized to determine the pathogenicity status of staphylococci. The significantly lower proportion of pathogenic staphylococci in the experimental group compared to the control group suggests a reduction in potential transmission rates. This, in turn, is anticipated to contribute to lower rates of healthcare-associated infections and consequently improve patient safety.

Catalase enzyme activity is used as a test for the identification of aerobic bacteria and certain facultative bacteria. In our study, the aerobic or facultative nature of bacteria present in the hand flora was determined. Samples were collected using both tape-stripping and swabbing methods from the experimental and control groups before and after contact. A significant presence of catalase-positive microorganisms was detected in these samples. However, after contact, there was a decrease in the proportion of catalase-positive microorganisms. In addition to biochemical tests that provide a general identification of microorganisms, the use of molecular techniques such as metagenomic analysis would offer a more precise characterization.

In this study, the microorganisms present in the general hand flora were identified simplistically, and the changes brought about by hand hygiene education were observed. Furthermore, the study shed light on the structure of hand flora after education in terms of microbial load. The results demonstrated experimental evidence that hand hygiene education leads to a reduction in hand microbiota load and a significant decrease in pathogenic groups. According to these results, hypothesis H1 is accepted.

Conclusion

In conclusion, reducing hand microbiota hand hygiene education is critical to preventing healthcare-associated infections, improving patient safety, and maintaining a sterile environment, which ultimately improves patient outcomes and overall healthcare quality.

Limitations

The most important limitation of the study is that the

study was carried out as a TUBITAK student project within the scope of 2209-A and the identification studies with molecular methods could not be carried out due to the budget limitations arising accordingly. The limited number of the study sample makes the generalizability of the results difficult. In addition, long-term results could not be evaluated due to time constraints. Another limitation is that since the main hypothesis of the study was to compare the hand flora of the participants, a post-test was not performed on the control group.

Conflict of Interest: The authors declare that there are no conflicts of interest.

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Ethics Committee Approval: This study was approved by the Bilecik Şeyh Edebali University Non-Interventional Clinical Research Ethics Committee with approval number 7/6 and date 22.11.2022.

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