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Isolation, Characterization, and Identification of Skin Microbiome Using Maldi-Tof Ms Among Microbiologists in a Non-Sterile Pharmaceutical Plant: Implication of Hand Hygiene Protocol Enhancement

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Abstract

The human skin microbiome is a complex ecosystem of microorganisms, including bacteria, fungi, and viruses, that play a vital role in maintaining skin health, regulating immune responses, and protecting against pathogens. While significant research has explored the microbiome's role in health and disease, work environments, particularly non-sterile pharmaceutical manufacturing plants, remain under-investigated. The non-sterile pharmaceutical plants are highly susceptible to microbial contamination, posing risks to product quality, regulatory compliance, and ultimately, patient safety and public trust. Microbiologists, through their skin microbiomes, may act as reservoirs or vectors for microbial transmission, emphasizing the need for detailed microbial profiling. Recent advancements have turned attention to the microbiome's potential to promote skin health and understanding of disease mechanisms. Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF MS) has emerged as a powerful, high-resolution, culture-independent tool for microbial identification. Hand hygiene practice is crucial in minimizing microbial cross-contamination, particularly in a non-sterile pharmaceutical industry where product safety, efficacy, and purity are important.

The study assessed the impact of a hand hygiene protocol by analyzing changes in microbial diversity, colony morphology, and transient bacterial load before and after its implementation. Microbial identification was carried out using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) at both stages. Statistical analyses, including percentages, means, and T-test, were used to evaluate the data and determine the protocol's effectiveness.

Results showed a significant reduction in microbial diversity, with alterations in colony morphology suggesting the elimination of certain bacterial species. However, MALDI-TOF MS identification results indicated no statistically significant difference in microbial composition. Notably, transient and environmental contaminants such as *Escherichia coli* and *Bacillus cereus* were eliminated post-hand hygiene implementation, reinforcing the protocol's efficacy in reducing contamination risks. On the contrary, an increase in coagulase-negative Staphylococcus species was observed, which is typically commensal and may pose a risk in pharmaceutical settings.

The persistence of unidentified isolates suggests limitations in microbial culture techniques and database constraints in MALDI-TOF MS. Overall, and the study emphasized the importance of stringent hand hygiene practices in controlling microbial contamination and maintaining pharmaceutical product safety, efficacy, and purity.

Keywords: *Skin Microbiome, Hand Hygiene, Non-sterile Pharmaceutical*

1. Introduction

The human skin microbiome is a complex ecosystem of microorganisms on the skin surface that has received much attention for its vital role in health and disease (Khurshid & Akash, 2024). The skin, a vital barrier against environmental threats, is home to various microbial communities, including bacteria, fungi, and viruses, which promote skin homeostasis, regulate immune function, and protect against pathogens. Recent studies have highlighted the impact of lifestyle, hygiene, and environmental exposures (Suwarsa et al., 2021). Nonetheless, work environments, particularly those with heightened microbial exposure, require further investigation in microbiome research. Pharmaceutical manufacturing plants provide a unique opportunity to examine the impact of workplace conditions on the human microbiome, especially in non-sterile environments characterized by numerous and unavoidable microbial interactions.

Non-sterile pharmaceutical plants are constantly changing environments where microbial presence is meticulously controlled but not entirely eradicated. In such environments, employees, especially microbiologists, are consistently exposed to various microbial populations via contact with raw materials, surfaces, air handling systems, and other personnel. Microbial contamination directly threatens product safety, regulatory compliance, and public trust within the pharmaceutical industry. Non-sterile manufacturing facilities, despite stringent quality control protocols, are vulnerable to microbiological variations from human sources. In these circumstances, the skin microbiomes of microbiologists serve as reservoirs or vectors for microbial transmission, highlighting the necessity of identifying these microbial communities. According to Yang et al. (2024), the skin microbiome is essential for the onset and progression of skin diseases, and its modulation is an effective therapeutic technique. Existing approaches encompass prebiotics and probiotics; however, the shortage of information regarding the skin microbiome hampers their successful application.

The skin microbiome is a vast and intriguing domain for researchers to investigate. The ongoing breakthroughs in science and technology present potential to enhance these investigations, yielding deeper and more comprehensive findings. Recent studies have focused on utilizing the skin microbiome to promote healthy skin. This is supported by a study conducted by Ito and Amagai (2023), which states that the skin microbiome is essential for homeostasis and the development of numerous illnesses. Investigating the roles of certain bacteria clarifies unknown aspects of disease pathogenesis and innovative treatment approaches, such as bacteriotherapy. However, the rapid advancement in the development of new probiotics poses several challenges for their clinical application, including long-term therapeutic efficacy, safety, and administration methods.

Despite its significance, not as much research has examined the occupational aspects of the skin microbiome, especially within the pharmaceutical industry. Current research has primarily concentrated on healthcare professionals or the general population, often neglecting the particular risks industrial personnel encounter. Other research focused on the gut microbiome has expanded to explore the role of microbiota in various physiological systems, including the skin. Moreover, conventional microbiological

techniques, such as culture-based methods, inadequately represent microbial diversity, as they fail to identify unculturable or low-abundance bacteria. The advancement of Matrix-Assisted Laser Desorption/Ionization Time-of-Flight mass spectrometry (MALDI-TOF MS) has transformed microbiome research by offering a high-resolution, culture-independent technique for detecting microbial communities. After utilizing MALDI-TOF MS, researchers surpassed traditional limitations and gained more significant insights into the composition of the skin microbiome.

This study sought to address the knowledge and research gap and investigated the skin microbiome of microbiologists in a non-sterile pharmaceutical plant using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) mass spectrometry. The objectives of this research were fourfold: to isolate and characterize skin microorganisms among microbiologists working in a pharmaceutical plant in Biñan, Laguna; to identify skin microorganisms using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) mass spectrometry; to determine the variety of microorganisms abundant on the skin (Dry Sites – Forearms and Hands); and to check the effectiveness of the hand hygiene protocol. The study aimed to enhance our understanding of the interplay between industrial environments and human microbiomes by providing a detailed microbial baseline for this occupational group. Furthermore, the findings could inform targeted interventions to optimize employee well-being and product safety in non-sterile pharmaceutical settings.

2. Methodology

This chapter outlines the research design and methodology, which served as the framework for the study's execution, and details the systematic approach employed to tackle the research problem. This included several components, such as the aims of the study, the research inquiries, the participants involved, the methods for data collection, and the procedures for data analysis. The document encompasses the research design, data sources, study population, instrumentation and validation, data collection, specimen collection, pre-analytical processing, laboratory procedures for the collected specimens, statistical analysis of the data, and waste disposal of the materials utilized in the study.

Sources of Data

The primary source of data for the study was the skin microbiome samples collected from the forearms and hands of thirteen (13) microbiologists working in a non-sterile pharmaceutical plant. Skin samples were collected from participants' left and right forearms and hands using the swab method to ensure a standardized approach. The collected samples were processed in the laboratory to isolate microbial cultures, which would then undergo morphological characterization. Following this, the isolates were purified. Finally, the isolates were identified at the species level using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) mass spectrometry, which provided detailed insights into the microbial composition of the participants' skin microbiome.

Preparation of Materials

Proper preparation of materials is essential for accurate and reliable microbiological testing. Glassware, such as test tubes, flasks, and beakers, was thoroughly cleaned and sterilized by autoclaving. Disposable Petri dishes were obtained in sterile conditions to prevent contamination. Sterile inoculating loops were used. Labels and marking instruments were systematically arranged to provide precise sample identification. Additionally, all materials were handled under aseptic conditions to maintain sterility and ensure the integrity of the experimental results.

Procedure

Sterilization

Sterilization is an essential procedure in microbiology laboratories, especially when it comes to the preparation of culture media. The established protocols were followed during sterilization to avoid contamination and to ensure that excessive heat did not affect other components. The biosafety cabinet designated for processing was sanitized before and after sample handling. It was essential to ensure that all glassware intended for use was adequately cleaned with a suitable detergent, thoroughly rinsed, and sterilized if necessary.

Collection of Skin Swab Samples

The participants' skin was sampled in a controlled laboratory setting by swabbing the microbiologists' forearms and hands with a sterile swab premoistened with phosphate buffer. The tip of the swab was aseptically cut and returned to the test tube. A 5 mL sterile phosphate buffer solution was added, and the cells were dislodged using a vortex mixer.

Isolation and Characterization of Bacterial Isolates

In duplicate, 1 mL of the phosphate buffer was transferred onto each sterile Petri dish. Twenty to twenty-five milliliters of sterile Tryptic Soy Agar were added, and the medium was allowed to solidify. The plates were incubated in an inverted position at 30–35°C for 72 hours. After incubation, the colonies were counted on each plate using a colony counter. Colonies exhibiting relatively high abundances were selected for morphological characterization based on their colony form, elevation, margin, surface, opacity, and pigmentation. These colonies were subcultured and purified on fresh sterile Petri dishes with Tryptic Soy Agar, followed by incubation at 30–35°C for 72 hours. The purified cultures were transported to another facility and processed for identification using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) mass spectrometry. Three microbiologists reviewed and confirmed the results to ensure accuracy and eliminate potential biases.

Matrix-Assisted Laser Desorption/IonizationTime-of-Flight (MALDI-TOF) mass spectrometry

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) is an analytical method that integrates matrix-assisted laser desorption/ionization (MALDI) with time-of-flight (TOF) mass spectrometry for the analysis of biomolecules. This approach involved initially combining the sample with a chemical matrix that facilitated desorption and ionization. A laser subsequently irradiated the sample-matrix mixture, resulting in the desorption and ionization of the analyte molecules. The produced ions were propelled into a time-of-flight analyzer, where they were distinguished according to their mass-to-charge (m/z) ratios. The duration for these ions to arrive at the detector was recorded, facilitating the calculation of their m/z values. The resultant mass spectrum yields a distinctive sample fingerprint applicable for identification and characterization (X. Chen et al., 2021).

Decontamination of Materials

After collecting the isolates, all materials were placed in an autoclavable PE bag and decontaminated with a dedicated autoclave for decontamination. This ensured that all materials are free from microorganisms that might cause contamination. The biosafety cabinet was also decontaminated by cleaning all surfaces using 70% alcohol.

Data Gathering Procedure

Data gathering for the study began with the creation of a letter of consent, ensuring ethical compliance and participant understanding. The target population, microbiologists employed by a pharmaceutical company, was provided with the informed consent form during in-person interactions to facilitate effective

communication and address any inquiries. Following the acquisition of consent, data collection commenced using the approved methods, ensuring minimal disruption to participants. Compliance with the Data Privacy Act was maintained by anonymizing participants through assigned codes instead of identifiable information. The collected data was securely stored and retained only until the completion and approval of the study, after which it was disposed of or archived responsibly.

This study also adhered to ethical guidelines to ensure the protection and privacy of all participants. Informed consent was obtained from each participant before sample collection, with clear communication regarding the purpose, procedures, and potential risks involved. Confidentiality of participant information was maintained throughout the study, and all data were anonymized to protect identities. All data will be stored for a period of one year. Participation was voluntary, and participants were given the right to withdraw at any stage without consequences. The study was conducted following institutional and regulatory ethical standards, ensuring that all procedures were designed to minimize discomfort and potential harm to participants.

Statistical Treatment of Data

The statistical tools used in this study to analyze and interpret the data are presented below:

1. Percentage was used to interpret the data collected on the morphological characteristics of the isolates and skin microbiome identification before and after the hand hygiene protocol.
2. Mean was used to determine the reduction in the number of microorganisms after the implementation of the handwashing protocol.
3. T-test was used to determine the significant difference in the isolated skin microorganisms before and after the implementation of the hand hygiene protocol.

Waste Disposal

In compliance with established waste disposal methods, all biological materials and laboratory waste produced during the research were managed and disposed of meticulously to mitigate environmental impact and ensure safety. Upon conclusion of the study, all disposable materials, such as used Petri dishes, pipette tips, and contaminated items, were collected and deposited into designated biohazard bins. These containers were properly marked and sealed to prevent leakage or spillage. Furthermore, all liquid waste, including bacterial cultures and media, was decontaminated prior to disposal. Ultimately, all waste materials were disposed of in accordance with local regulations pertaining to biohazardous waste and chemical management within the manufacturing facility. By adhering to these waste disposal protocols, the researcher ensured the safe and responsible handling of laboratory waste related to the skin microbiome investigation, thus avoiding potential threats to human health, the environment, and finished products.

3. Presentation, Analysis, and Interpretation of Data

This chapter presents the results of the analysis and interpretation of the data obtained from the investigation. The issues that were discussed in the first chapter served as a compass for the analysis and interpretation that followed.

Morphological characteristics of the isolated skin microbiome before and after hand hygiene protocol implementation

Table 1 Morphological Characteristics of the Isolates

Pre Test											
Form		Elevation		Margin		Surface		Opacity		Pimentation	
Circular	78.42%	Raised	55.40%	Entire	100.00%	Glistening	48.20%	Opaque	99.28%	Orange	0.72%
Irregular	8.63%	Convex	11.51%	Unduate	0.00%	Smooth	39.57%	Translucent	0.72%	Yellow	23.02%
Punctiform	12.95%	Flat	32.37%	Filiform	0.00%	Dull	12.23%			White	61.87%
		Umbonate	0.72%	Curled	0.00%					Light Yellow	0.72%
		Crateriform	0.00%	Lobate	0.00%					Cream	11.51%
										Peach	1.44%
										Pink	0.72%

Post-Test											
Form		Elevation		Margin		Surface		Opacity		Pimentation	
Circular	83.33%	Raised	27.78%	Entire	92.59%	Glistening	42.59%	Opaque	81.48%	Orange	0.00%
Irregular	3.70%	Convex	0.00%	Unduate	0.00%	Smooth	22.22%	Translucent	18.52%	Yellow	14.81%
Punctiform	12.96%	Flat	64.81%	Filiform	0.00%	Dull	35.19%			White	42.59%
		Umbonate	7.41%	Curled	7.41%					Light Yellow	3.70%
		Crateriform	0.00%	Lobate	0.00%					Cream	16.67%
										Peach	5.56%
										Pink	0.00%
										Off White	7.41%
										White to Cream	9.26%

The data on the morphological characteristics of bacterial colonies isolated from the skin microbiome before and after the hand hygiene protocol presented significant shifts in microbial diversity and abundance. Table 1 presents the morphological characteristics prior to hand hygiene. The dominant colony form was circular at around 78.42%, followed by punctiform at 12.95% and irregular at 8.63%. After hand hygiene, circular colonies remained dominant but increased in proportion to around 83.33%, while irregular colonies saw a marked decline to around 3.70%, suggesting that hand hygiene effectively reduced the presence of less common morphologies.

Elevational characteristics also shifted, with raised colonies decreasing from 55.40% to 27.78%, while flat colonies increased from 32.37% to 64.81%, possibly indicating a reduction in more resilient or structured biofilm-forming bacteria. In terms of margin, entire margins remained predominant but slightly decreased, while curled margins emerged post-wash at 7.41%, which could be due to the selective survival of certain strains.

Regarding surface characteristics, the glistening surface showed a slight decrease from 48.20% to 42.59%, while smooth textures decreased from 39.57% to 22.22%, and dull surfaces increased from 12.23% to 35.19%, possibly reflecting a shift in microbial composition toward species that tolerate environmental stress. A noteworthy change was observed in opacity, where translucent colonies increased from 0.72% to 18.52%, suggesting that some bacteria with thinner or less pigmented cell structures persisted despite hand hygiene.

Pigmentation patterns also underwent notable changes. White colonies, the most prevalent before hand hygiene at 61.87%, decreased post-wash to 42.59%, while yellow and orange colonies diminished substantially. Meanwhile, less common pigmentation patterns, such as off-white and white-to-cream, emerged post-wash, potentially indicating a shift toward more resistant or resident skin microbiota.

After the hand hygiene protocol, circular colonies remained the dominant form, comprising approximately 83.33%. Flat colonies were the most prevalent in terms of elevation, accounting for 64.81%. Regarding surface characteristics, glistening colonies were the most common, making up 42.59%. Opaque colonies continued to dominate, representing 81.48% of the total. Lastly, in terms of pigmentation, white colonies remained the most prevalent at 42.59%.

In terms of morphological characteristics, the efficacy of hand hygiene significantly reduced the overall bacterial diversity and shifted the microbial profile toward species that may be more resilient to cleaning.

These results emphasize the importance of proper hand hygiene in minimizing transient microbial contamination while potentially allowing more resident microbiota to persist. As discussed in the study conducted by De Almeida & Borges et al. (2007), which was cited by Zapka et al. in 2017, the reduction in less common morphologies may also be due to these bacteria's lower resilience to the mechanical and chemical actions involved in hand hygiene. Common skin flora, such as *Staphylococcus epidermidis*, are well-adapted to the skin environment and may possess mechanisms to withstand such interventions better than less prevalent species.

However, bacterial morphology alone is not sufficient to accurately identify the bacteria that colonized the skin. While morphological characteristics such as form, elevation, margin, surface, opacity, and pigmentation provide useful preliminary information, they still lack the specificity required for definitive identification and, in turn, for concluding that the hand hygiene protocol has been effective. In *Bailey & Scott's Diagnostic Microbiology* by Tille (2024), it was noted that many unrelated bacteria shared the same morphological characteristics and required further biochemical tests for differentiation. Additionally, Madigan et al. (2020) mentioned that some bacteria exhibit pleomorphism, meaning they can change their shape under different environmental conditions, making morphology an unreliable standalone and inconclusive tool.

Commonly identified organisms in the skin microbiome before hand hygiene protocol implementation using MALDI-TOF mass spectrometry.

Figure 2.1 Skin Microbiome Identification Before Hand Hygiene Protocol

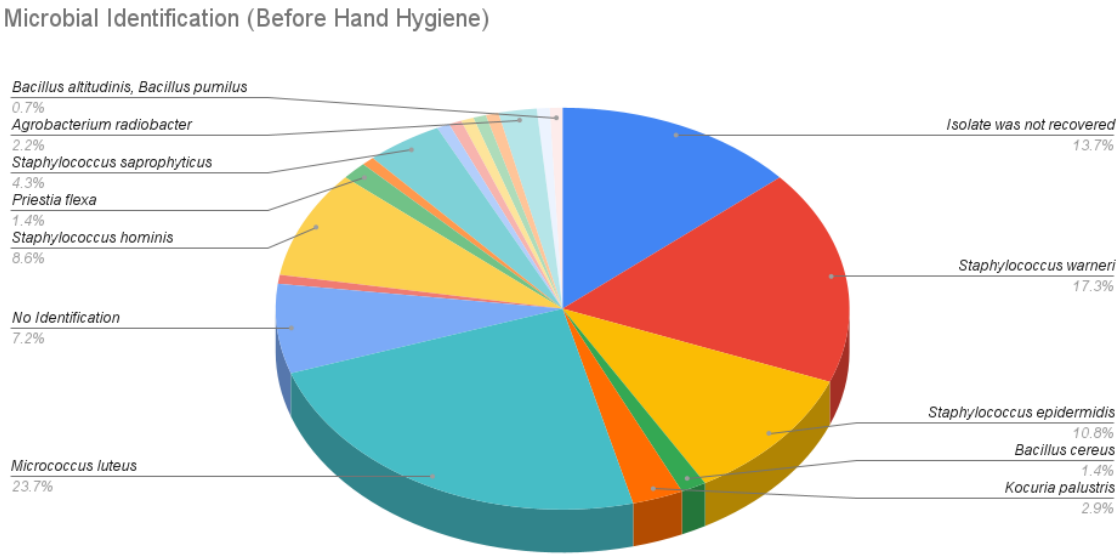

Figure 2.1 presents the distribution of microorganisms identified on the skin microbiome of analysts before the implementation of the hand hygiene protocol. The most prevalent species was *Micrococcus luteus* at 23.7%, a common skin commensal microorganism known for its role in maintaining microbial homeostasis. *Staphylococcus warneri* at 17.3% and *Staphylococcus epidermidis* at 10.8% were also prominent among the total isolated bacteria. Both microorganisms are coagulase-negative staphylococci that are typically non-pathogenic but can be pathogenic in immunocompromised individuals. A case study performed by Alawad et al. (2022) detailed an instance of native mitral valve endocarditis caused by *S. warneri* in an immunocompetent host. The study highlighted the pathogenic potential of *S. warneri*, even

among individuals without traditional risk factors, such as immunosuppression or recent surgical procedures. This finding was further supported by a study conducted by Louail et al. (2023), which investigated the invasive potential of *S. warneri* within the gut microbiota. The study presented evidence that *S. warneri* could invade intestinal cells, suggesting a potential role in gut-related infections, especially in patients with compromised immune systems. On the other hand, *S. epidermidis*, though generally non-pathogenic, has been implicated in various infections, especially among immunocompromised patients. Its ability to form biofilms on medical devices makes it a notable pathogen in healthcare settings.

Other microorganisms were also identified in smaller amounts, including *Staphylococcus hominis* at 8.6%, *Staphylococcus saprophyticus* at 4.3%, and *Kocuria palustris* at 2.9%. As stated by Severn et al. (2022), Rafiee and Ghaemi (2023), and Varghese et al. (2023) in their respective studies, these three microorganisms are part of the normal skin flora. Uncommon microorganisms, such as *Agribacterium radiobacter* at 2.2% and *Priestia flexa* at 1.4%, were also detected. These microorganisms are commonly isolated from the environment and could be potential environmental contaminants, as mentioned by Namdari et al. (2003) and Soto-Varela et al. (2024) in their studies.

Additionally, *Bacillus cereus* at 1.4% and *Escherichia coli* at 0.7% were also isolated from the skin microbiome sample. *B. cereus* is associated with foodborne illness (McDowell et al., 2023), while *E. coli*, commonly found in the intestines of humans and animals, plays a role in gut health (Ramos et al., 2020). While most strains are harmless, some pathogenic strains can cause foodborne illnesses, urinary tract infections, and other infections (About Escherichia Coli Infection, 2024). These organisms may represent transient contamination, but they pose a contamination risk to non-sterile products being tested if they come into contact with the samples.

Interestingly, 13.7% of the total samples were not recovered, and 7.2% could not be identified, which may be due to limitations in microbial culture techniques and the database of MALDI-TOF-MS (Calderaro & Chezzi, 2024).

Figure 2.2 Skin Microbiome Identification After Hand Hygiene Protocol

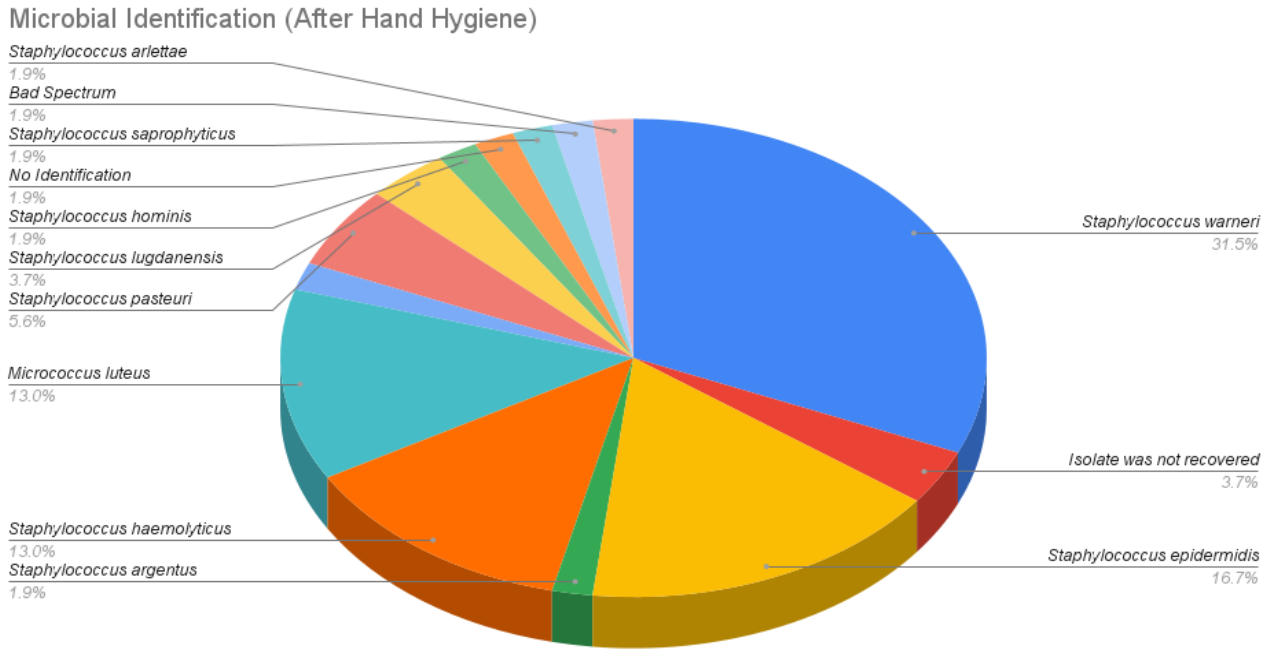

Figure 2.2 presents the skin microbiome identification after the implementation of the hand hygiene protocol. The most prevalent bacterial species identified post-hand hygiene was *Staphylococcus warneri*, accounting for 31.5% of the total microbial population identified. *Staphylococcus epidermidis* was the second most abundant, making up 16.7%, followed by *Micrococcus luteus* and *Staphylococcus haemolyticus*, each at 13.0%. Other bacteria detected in smaller amounts included *Staphylococcus pasteuri* at 5.6%, *Staphylococcus lugdunensis* at 3.7%, and *Staphylococcus arlettae* at 1.9%. All the organisms identified reside on the skin; some isolates, such as *Micrococcus luteus*, can also be isolated from environmental sources such as soil, water, and air (Zhu et al., 2021).

Compared to the results of the pre-hand hygiene protocol, the percentage of samples where no microbial isolates were recovered was relatively low, around 3.7%, which may indicate that the hand hygiene protocol likely altered the microbial composition but did not completely eliminate the normal skin flora. It is also noteworthy that *Bacillus cereus* and *Escherichia coli* were no longer isolated or identified. A notable shift in bacterial diversity was observed when compared to the pre-hygiene isolates, suggesting that hand hygiene selectively reduces certain bacteria, including opportunistic and potentially pathogenic species like *B. cereus* and *E. coli*. On the other hand, *S. warneri* and *S. epidermidis* remained prevalent. This may indicate the resilience of some commensal bacteria to hand hygiene practices. The commensal or normal flora of the skin has two main protective functions: microbial antagonism and competition for nutrients in the ecosystem (WHO Guidelines on Hand Hygiene in Health Care, 2009). The shift in bacterial prevalence may suggest that while hygiene practices effectively reduce microbial load, they may also influence the alteration of the microbial community structure of the skin.

Reduction of the Number of Microorganisms After the Hand Washing Protocol

Table 2 Log reduction of Microbial Count Before and After Hand Hygiene

	Mean	Log reduction	Interpretation
Before	100.769	0.0477	Not Significant
After	90.404		

Table 2 presents the microbial log reduction after the implementation of the hand hygiene protocol. The mean microbial count before hand hygiene was 100.769, which slightly decreased to 90.404 after the protocol was implemented. This resulted in a 0.0477 log reduction in microbial load. The results suggested a minimal decrease in microbial presence, which was deemed not statistically significant, indicating that the handwashing protocol was ineffective in reducing the microbial count.

A 1-log reduction corresponded to a 90% reduction in microbial count, meaning the bacterial population was reduced to one-tenth of its original count (Tanner, 2024). However, a 0.0477 log reduction was much smaller, representing only about a 1% reduction in microbial count—an almost negligible change. These findings suggested that the current hand hygiene method used in the study might not have been sufficient to significantly reduce microbial counts.

Significant Difference in the Isolated Skin Microbiome Before and After Hand Hygiene Protocol

Table 3 Paired Sample Test of Morphological Characteristics

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	preform - postform	28.33333	30.89229	17.83567	-48.40736	105.07403	1.589	2	.253
Pair 2	preelevation - postelevation	17.00000	26.28688	11.75585	-15.63947	49.63947	1.446	4	.222
Pair 3	premargin - postmargin	17.00000	40.28647	18.01666	-33.02226	67.02226	.944	4	.399
Pair 4	presurfaces - postsurfaces	28.33333	26.27420	15.16941	-36.93539	93.60205	1.868	2	.203
Pair 5	preopacity - postopacity	42.50000	72.83200	51.50000	-611.86954	696.86954	.825	1	.561
Pair 6	prepimentation - postpimentation	13.42857	23.57864	8.91189	-8.37804	35.23518	1.507	6	.183

The table 3 presents the results of a paired t-test assessing differences in morphological characteristics before and after the hand hygiene protocol. The p-values (Sig. 2-tailed) for all six pairs were greater than 0.05, indicating that none of the differences were statistically significant. Thus, we failed to reject the null hypothesis, meaning that the changes in form, elevation, margin, surface, opacity, and pigmentation could have occurred by chance. The 95% confidence interval included zero, which further suggested that there was no significant reduction in microbial diversity in terms of morphological characteristics. The statistical analysis did not support a conclusive effect of the hand hygiene protocol on microbial colony morphology.

Across all morphological characteristics (form, elevation, margin, surface, opacity, and pigmentation), there was a noticeable reduction after the hand hygiene implementation. This implied that the intervention effectively reduced microbial diversity and altered colony morphology, possibly due to the elimination of certain bacterial species or changes in their growth dynamics.

As presented in Figures 2.1 and 2.2, the implementation of the hand hygiene protocol resulted in a significant change in the composition of the skin microbiome among the analysts who were sampled. Therefore, we rejected the null hypothesis. Before the protocol was enforced, *Micrococcus luteus* was the most prevalent microorganism, accounting for around 23.7% of the total isolates. This was followed by *Staphylococcus warneri* (17.3%) and *Staphylococcus epidermidis* (10.8%), both of which are coagulase-negative *Staphylococcus* species commonly found on human skin. Environmental contaminants such as *Agrobacterium radiobacter* and *Priestia flexa* were also identified, suggesting possible exposure to non-sterile surfaces in the pharmaceutical setting. In addition, transient bacteria such as *E. coli* and *B. cereus* were also detected, which could have raised concern about potential contamination risks in laboratory environments and, in turn, the products being tested.

After the implementation of the hand hygiene protocol, notable changes were observed. *Staphylococcus warneri* emerged as the most dominant species, increasing to 27.1%, followed by *Staphylococcus epidermidis* at 18.8% and *Staphylococcus haemolyticus* at 13.5%. The reduction of *Micrococcus luteus* and the absence of *E. coli* and *B. cereus* indicated that proper hand hygiene effectively minimized transient and potentially pathogenic bacteria.

While the presence of pathogenic bacteria decreased, there was an increase in coagulase-negative *Staphylococcus* species, including *Staphylococcus haemolyticus*, *Staphylococcus pasteuri*, and *Staphylococcus lugdunensis*. While these bacteria are typically harmless commensals, they can become

opportunistic pathogens in immunocompromised individuals. Another observation from the data was the persistence of non-recovered and unidentified isolates, which may have been attributed to limitations in microbial culture techniques and the MALDI-TOF MS database constraints, as mentioned in a study by Sung et al. (2017) and cited by Roux-Dalvai et al. (2019).

The data suggested that hand hygiene was effective in reducing transient and environmental contaminants while favoring the persistence of normal skin flora. This change was crucial in a pharmaceutical setting, where microbial contamination could pose a significant risk to product safety (Microbial Control of Raw Materials Used in Pharmaceuticals, 2022). The result emphasized the need for stringent hand hygiene protocols to maintain a controlled microbiome and minimize potential contamination sources.

The implementation of a stringent hand hygiene protocol was essential not only for microbiologists in the QC Laboratory but also for all personnel involved in pharmaceutical manufacturing, from raw material dispensing to production and packaging. The microorganisms identified served as a general representation of the skin microbiota, which could have been at risk of microbial transfer to pharmaceutical products. Prior to hand hygiene, the most prevalent species were *Micrococcus luteus* and *Staphylococcus warneri*, whereas post-hand hygiene, *S. warneri* was dominant, followed by *S. epidermidis*. These microorganisms, commonly found on human skin, were frequent contaminants in cleanroom environments and had the potential to be shed from personnel onto pharmaceutical products.

The presence of *M. luteus*, *S. warneri*, and *S. epidermidis* in manufacturing areas posed a significant threat to product quality, integrity, and purity. *M. luteus* could withstand nutrient-scarce environments, while *S. warneri* and *S. epidermidis* could develop resistance to antibiotics, complicating treatment options resulting from contaminated pharmaceutical products. If not effectively controlled, microbial contamination could lead to compromised product safety, regulatory non-compliance, and potential health risks to consumers. Such contamination could result in costly product recalls, reputational damage, and financial losses for the company. The data gathered suggested a critical need for continuous monitoring, reinforced hygiene practices, and personnel training to mitigate contamination risks and ensure that products released to the market were safe, pure, and effective.

Table 4 Difference in Isolated Skin Microbiome Before and After Hand Hygiene Protocol in terms of Microbial Count

	Mean	t-Test	p-value	Interpretation
Before	100.769	0.357	0.722	Not Significant
After	90.404			
Significance level @ 0.05				

Table 4 presents the statistical result using a t-test to compare the microbial count of the skin before and after the hand hygiene protocol. The mean microbial count before the hand hygiene protocol was 100.769. On the other hand, the mean microbial count after the hand hygiene protocol was 90.404. The paired t-test was used to determine whether the reduction of microbial count was statistically significant. The t-test value was 0.357, with a corresponding p-value of 0.722. Since the p-value was greater than the significance level of 0.05, the result could be interpreted as not statistically significant; therefore, the null hypothesis was accepted. There was no strong evidence that the hand hygiene protocol caused a significant reduction in microbial count.

Based on the table, although there was a numerical decrease in the microbial count after following the hand hygiene protocol, the statistical results indicated that the decrease was not significant at the 0.05 level of significance. The hand hygiene protocol without the use of any disinfectant did not have a statistically measurable effect on reducing skin microbial count.

Improvements and recommendations for the current hand hygiene protocol for microbiologists in a non-sterile pharmaceutical plant

Based on the results of the study, it can be deduced that the current hand hygiene practices among the microbiologists in the non-sterile pharmaceutical plant are not entirely effective in eliminating transient contaminants and possible pathogenic microorganisms. The presence of high residual microbial load after the hand hygiene procedures, without the use of any disinfectants, may suggest gaps in techniques, product efficacy or compliance with the established protocol. However, the current hand hygiene protocol may provide a baseline in terms of safety but is insufficient to fully address contamination risks. The variability in microbial load before and after hand hygiene stresses the need to enhance the protocol that emphasizes proper handwashing techniques, the use of more effective antimicrobial agents, and increased monitoring to ensure consistent compliance. The findings of the study emphasize the importance of reinforcing the hand hygiene protocol to improve microbial reduction and minimize potential cross-contamination risks in the pharmaceutical plant environment.

Table 5 Possible Problems with Hand Hygiene Protocol and Suggestions for Enhancements

Possible Problems with the Hand Hygiene Protocol	Suggestions for Enhancements
Insufficient frequency of hand hygiene	Hand washing is not mandated at critical control points (e.g. before entering the testing area). Introduction of a more structure schedule, assigning hand washing station upon entry, handwashing before and after key tasks or possible microbial contamination event.
Inadequate detergent and disinfectant formulation	The current detergent and antimicrobial product may not be effective against specific contaminants, such as gram-positive and spore-forming bacteria. Evaluating and validating the detergent and the hand hygiene products to check the efficacy against a broad spectrum of microorganisms, including but not limited to the two spectra that was already mentioned.
No standardization in disinfectant usage	The use of antimicrobial agents is not explicitly stated into the protocol. Specific guidelines on when and how to use antimicrobial agents as a secondary measure to handwashing.
Absence of post-activity review	Hand hygiene can be a performance indicator. Introduction of periodic microbial testing post-handwashing to evaluate the effectiveness of the protocol and adjust methods if needed.

Lack of consistency in handwashing techniques	The microbiologists may not be following the proper technique. It would be necessary to provide mandatory periodic training on proper handwashing techniques, which might include friction, drying and the duration of hand washing

4. Results and Discussion

This study adopted an experimental research approach to isolate, characterize, and identify the skin microbiome using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) mass spectrometry among microbiologists in a non-sterile pharmaceutical plant which will be used for hand hygiene protocol enhancement. This study examined the relationship between morphological characteristics of microbial isolates and the microbial identity and hand hygiene practices of microbiologists working in non-sterile pharmaceutical facilities.

Summary of Findings

This study adopted an experimental research approach to isolate, characterize, and identify skin microbiome using MALDI-TOF Mass Spectrometry Among Microbiologists in a Non-Sterile Pharmaceutical Plant: Implication for Hand Hygiene Protocol Enhancement. Samples were taken from thirteen (13) microbiologists working in the pharmaceutical plant and all signed an informed consent. The following is a summary of findings based on the analysis of data gathered.

After the hand hygiene protocol, circular colonies remained the dominant form, comprising approximately 83.33%. Flat colonies were the most prevalent in terms of elevation, accounting for 64.81%. Regarding surface characteristics, glistening colonies were the most common, making up 42.59%. Opaque colonies continued to dominate, representing 81.48% of the total. Lastly, in terms of pigmentation, white colonies remained the most prevalent at 42.59%.

The percentage distribution of bacterial isolates before the hand hygiene protocol revealed that *Micrococcus luteus* was the most prevalent species, comprising approximately 22.33% of the total isolates. This was followed by *Staphylococcus warneri,* accounting for 18.45%, and *Staphylococcus epidermidis*, which made up 13.59% of the identified bacteria. *Staphylococcus hominis* constituted 10.68% of the total. A smaller but notable presence of unidentified bacteria (6.80%) was observed. *Staphylococcus saprophyticus* (4.85%) and *Kocuria palustris* (3.88%) were also detected, albeit in lower proportions. Less frequently isolated bacteria included *Bacillus cereus* (1.94%), *Priestia flexa* (1.94%), and *Agrobacterium radiobacter* (2.91%).

Rare isolates, each making up less than 1% of the total, included P*riestia megaterium, Metabacillus idriensis, Pseudomonas stutzeri, Staphylococcus aureus spp. aureus, Staphylococcus ureilyticus, Staphylococcus haemolyticus, and E. coli.* Additionally, a single instance of *Bacillus altitudinis* and *Bacillus pumilus* was identified.

Following the hand hygiene protocol, *Staphylococcus warneri* remained the most frequently isolated species, accounting for 24.56% of the total microbial population. *Staphylococcus epidermidis* was the second most common, making up 15.79%, while both *Staphylococcus haemolyticus* and *Micrococcus luteus* each represented 10.53% of the total isolates.

Other notable bacterial species included *Staphylococcus pasteuri* (5.26%), *Staphylococcus lugdanensis* (3.51%), and *Staphylococcus hominis, Staphylococcus saprophyticus, Staphylococcus argentus, Staphylococcus arlettae, and Priestia megaterium,* each contributing 1.75% to the overall microbial

population. Additionally, a small proportion of isolates were classified as "No Identification" or "Bad Spectrum", indicating inconclusive results.

A 0.0478 log reduction was much smaller, only about a 1% reduction in microbial count - an almost negligible change. The result suggests that the current hand hygiene method used in the study might not be sufficient to reduce microbial count significantly.

Across all morphological characteristics (form, elevation, margin, surface, opacity, and pigmentation), there was a noticeable reduction after hand hygiene implementation. This implies that the intervention effectively reduced microbial diversity and altered colony morphology, possibly due to the elimination of certain bacterial species or changes in their growth dynamics. The findings highlight the effectiveness of hand hygiene in modifying microbial colony characteristics, reinforcing its importance in infection control and microbiome management.

Identified microorganisms of pre and post-hand hygiene protocol were compared, and a notable shift in bacterial diversity was observed, suggesting that hand hygiene selectively reduces certain bacteria including those that can be opportunistic pathogen and possibly pathogenic such as *B. cereus* and *E. coli*. On other hand, *S. warneri* and *S. epidermidis*, remained prevalent. This may indicate the resilience of some commensal bacteria to hand hygiene practices. The commensal or the normal flora of the skin has two main protective functions: microbial antagonism and the competition for nutrients in the ecosystem. (*WHO Guidelines on Hand Hygiene in Health Care*, 2009) The shift in bacterial prevalence may suggest that while hygiene practices effectively reduce microbial load, they may also influence the alteration of microbial community structure of the skin.

The mean microbial count before hand hygiene was 100.769, which slightly decreased to 90.404. After the implementation, the result was a 0.0477 log reduction of microbial load. The results suggest a minimal decrease in microbial presence, which was deemed not statistically significant, and the handwashing protocol was ineffective in reducing microbial count.

Microbiologists working in a non-sterile pharmaceutical plant are in charge of testing the finished good to check for microbial integrity prior to market release. It is integral that they practice proper hand hygiene practice and ensure that they adhere to it on top of following aseptic techniques. After data collection and analysis, there is a need to propose enhancements to the existing hand hygiene protocol for microbiologists to maintain a safe and sanitary environment in a non-sterile pharmaceutical.

5. Conclusions and Recommendations

Hand hygiene effectively reduces bacterial diversity and shifts the microbial profile toward more resilient species, emphasizing its role in minimizing transient contamination while allowing the resident flora to persist. This provides preliminary information, but bacterial morphology lacks the specificity needed for accurate identification and to fully assess the hand hygiene protocol’s effectiveness.

MALDI-TOF MS effectively identifies microbial isolates but shows limitations in identifying some species due to poor spectrum quality, limited database, or similarities in their protein profiles. Hand hygiene implementation significantly reduces transient microorganisms but has little impact on resilient commensal microbiomes, with Staphylococcus warneri and Staphylococcus epidermidis remaining dominant post-intervention.

Although there is a numerical decrease in the microbial load, the hand hygiene practice is deemed ineffective in reducing commensal microorganisms. However, it can eliminate transient microorganisms that colonize the superficial layer of the skin, potentially causing contamination to both the analysts and

the product being tested.

The hand hygiene protocol significantly reduces microbial diversity and alters colony morphology, suggesting an effect on the microbial composition. MALDI-TOF MS shows no significant change in microbial identification, but the protocol reduces transient contaminants like E. coli and B. cereus. However, an increase in Staphylococcus species highlights a potential risk to product integrity and, in turn, patient safety. The low 0.0477 log reduction indicates that while the protocol is effective in reducing transient microorganisms, it may not fully eliminate them.

It is essential to include enhancements to the existing hand hygiene protocol for microbiologists to maintain a safe and sanitary environment in a non-sterile pharmaceutical plant. Reinforcing the protocol may further reduce transient microbial contamination and improve overall product safety, integrity, and efficacy.

Given the results of this study, the researcher provides five (5) recommendations as the basis for the improvement of the hand hygiene protocol and future research:

Reinforce Hand Hygiene Compliance and Protocol Refinement: Given that bacterial isolates were still detected, whether transient or commensal, the researcher recommends reinforcing hand hygiene compliance among analysts. The Human Resources Department should include periodic training on proper hand washing techniques to ensure adequate contact time with disinfectants or antimicrobial agents and ensure stricter adherence to the hand hygiene protocol in the non-sterile plant.

For Microbiologists and Future Researchers: MALDI-TOF MS is a valuable tool for bacterial identification, but its limitations in detecting and differentiating certain microorganisms would require the use of additional methods for microbial identification. It would still be better to consider the gold standard in microbial identification, which is 16S rRNA genome sequencing or PCR-based identification, to increase the accuracy of microbial profiling.

Microbial Surveillance in the QC Microbiology Laboratory: The QC Microbiology laboratory should start microbial surveillance to assess the effectiveness of the hand hygiene protocol over time. This can include continuous sampling of the skin, air, water, and surfaces to identify any possible sources of contamination. Improvements should also include environmental controls such as hand and surface disinfection, which will help minimize bacterial contamination and, in turn, reduce the log to an acceptable level.

Future Research: Future researchers could use this study as a starting point and include additional sampling sites and increase the number of respondents from different manufacturing facilities, including sterile and cosmetics plants. The additional sampling sites would reveal other types of microorganisms. Future researchers should also explore the antimicrobial resistance profiles to expand the knowledge and evaluate the efficacy of alternative hand sanitization methods to reduce microbial load further and prevent contamination in non-sterile pharmaceutical settings.

Ongoing Monitoring and Training: The enhanced hand hygiene protocol should be implemented with ongoing monitoring to identify future opportunities for improvement. Periodic training sessions and refresher courses should be conducted to reinforce proper hand hygiene techniques. To supplement the training, periodic microbial load assessments and feedback mechanisms would help identify future gaps and ensure sustained compliance.

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