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The Effectiveness of Different Cleaning Methods on the Microbial Load of Reusable Menstrual Pads

***[1]Rajah, A. S., [1]Yunusa, U., [2]Tashi, F, T., [3]Tukuntawa, A. S. and [2]Abdullahi, S. O.**

[1]Department of Nursing Science, Faculty of Allied Health Sciences, Bayero University, Kano, Nigeria

[2]Department of Nursing, College of Nursing and Midwifery, Kano, Nigeria.

[3]School of Post Basic Nursing, National Orthopaedic Hospital, Dala-Kano, Nigeria

Correspondence: asrajah.nur@buk.edu.ng; +2347037550409

Abstract

Menstrual pads are essential hygiene products used to manage menstrual flow, ensuring comfort and dignity for menstruating individuals. Reusable menstrual pads are re-gaining popularity as a sustainable and cost-effective alternative to disposable ones. This study aimed to determine the efficacy of various cleaning methods in reducing the microbial load of reusable menstrual pads. A total of 26 samples from reusable menstrual pads were collected. The participants were directed to follow specific washing protocols as highlighted in recommendations by UNICEF (washing with water only, detergent only, bar soap only, detergent and disinfectant and detergent, disinfectant, and ironing). Samples (used reusable menstrual pads) were retrieved from participants to analyze any microbial presence on the pads themselves. Microbiological analysis was conducted to assess microbial growth on nutrient agar, chocolate agar, and Sabouraud dextrose agar. Results obtained showed low microbial load in 53.8% of the samples analyzed. All reusable menstrual pads (RMPs) washed with water only (5) were positive for bacterial culture with a count of > 100cfu/ml. Those washed with Viva detergent and May bar soap revealed a microbial count of 50-100cfu/ml. RMPs washed with Viva detergent and Dettol disinfectant, dried, and then ironed showed no significant growth or very low microbial counts compared to other cleaning methods (p-values: mesophilic bacteria, p = 0.009; molds, p = 0.009; anaerobic bacteria, p = 0.026; and Staphylococcus spp., p = 0.001). The predominant isolates are Staphylococcus species (26.9%), Lactobacillus species (26.9%), Candida species (19.2%), and Fusobacterium nucleatum (23%). The study revealed that reusable menstrual pads cleaned with water only had the highest microbial loads, while those cleaned with a combination of detergent, disinfectant, and ironing showed minimal to no microbial contamination, demonstrating this method as the most effective for ensuring hygiene and safety.

Keywords: Bacterial burden, Cleaning methods, Disinfection, Fungal load, Menstrual hygiene, Reusable menstrual pads,

INTRODUCTION

Menstruation is a natural and healthy cycle in fertile women and girls from puberty to menopause, with approximately 1.8 billion women globally experiencing it monthly (Anaba *et al.*, 2022). Ensuring optimal hygiene practices during menstruation is essential for preventing associated infections (Warashinta *et al.*, 2021). However, a staggering 500 million women and girls worldwide lack access to menstrual hygiene facilities, including safe and hygienic menstrual management materials(Anaba *et al.*, 2022). Additionally, managing menstruation effectively and hygienically can be a significant challenge, especially in low-income settings (Hennegan *et al.*, 2017). Limited access to clean water, sanitation facilities, and appropriate menstrual products often leads to negative health consequences, hinders education opportunities, and reduces overall well-being (UNICEF, 2020). Providing safe and respectful ways to manage menstruation (often referred to as Menstrual Hygiene Management or MHM) is essential for women's overall health and well-being (Hand *et al.*, 2023). Having access to safe and dignified menstruation is a fundamental right for all women and girls (UNICEF, 2020). UNICEF's vision is a world where every girl can go about her daily activities – learning, playing, and taking care of herself – without the added stress, shame, or restrictions associated with menstruation due to lack of information or supplies (UNICEF, 2020). Growing evidence

based on low- and middle-income countries shows that many girls are not able to manage their menses and associated hygiene with ease and dignity(Asumah *et al.*, 2022; Kumie *et al.*, 2022).

This deprivation is even more alarming for girls and women in emergencies.

Properly managing menstruation involves the availability of absorbent materials and the provision of safe, accessible, and private sanitation facilities alongside culturally appropriate information on menstrual hygiene management (MHM)(Hand *et al.*, 2023). Despite global advancements in menstrual health awareness and the proliferation of products, many women in resource-limited settings still rely on reusable menstrual pads (RMPs) due to their cost-effectiveness and environmental sustainability (Shibly *et al.*, 2021).

RMPs are a type of menstrual product worn externally to the body in the underwear, to absorb menstrual flow and held in place usually by snaps. They are made from natural or synthetic materials (UNICEF, 2020). The materials used in RMPs, such as organic cotton, bamboo, and other eco-friendly fabrics, are gentle on the skin, highly absorbent, and free from harmful chemicals often found in disposable pads. After use, they are washed, dried, and re-used (UNICEF, 2020). RMPs are re-gaining popularity as a sustainable and cost-effective alternative to disposable options. These pads are designed to be durable and can be used repeatedly over a long period, which makes them an economical choice for women and girls, especially in low-income countries (World Bank Group, 2023).

Despite these benefits, the potential for microbial growth on RMPs raises concerns about user safety and infection risks. The unique environment created by menstrual blood and the potential for improper cleaning practices could lead to the proliferation of bacteria and fungi, potentially causing urinary tract infections (UTIs), yeast infections, and other health complications (Anderhell & Sundberg, 2019).

Additionally, the use of RMPs poses potential health risks if not properly managed. Inadequate washing and drying practices can lead to the proliferation of bacteria and other pathogens, potentially increasing the risk of urinary tract infections, vulvovaginitis, and other reproductive health issues (Anderhell & Sundberg, 2019). The importance of evaluating the microbial safety of these products cannot be overstated, particularly as their use is advocated as a sustainable option in both developing and developed countries (UNICEF, 2020).

Recent studies have revealed significant knowledge gaps in the proper care and microbial safety of reusable menstrual pads (RMPs), emphasizing the lack of research on effective cleaning methods to reduce microbial growth and health risks, particularly in low- and middle-income countries (Asumah *et al.*, 2022; Parikh & Nagar, 2022; Pednekar *et al.*, 2022; Roxburgh *et al.*, 2020). This study addresses these gaps by evaluating microbial burden, infection risks, and the effectiveness of cleaning methods for RMPs, providing actionable guidelines to promote safer use and support menstrual health equity.

MATERIALS AND METHODS

Study Design

This study employed an *in vitro* (laboratory-based) approach to evaluate the microbial burden associated with RMPs and assess the effectiveness of different cleaning methods in mitigating microbial growth.

Study Population

All participants in the present study were healthy women 18–45 years old who resided in Kano, Nigeria, had a menstrual cycle of 21–30 days, and were menstruating (day 2, 3, or 4 of the menstrual cycle) at the time of sampling. The study excluded pregnant or lactating women, women with a history of pelvic inflammatory disease or recurrent UTIs, and women using any vaginal medications within the past month. Informed consent was obtained from all participants before sample collection. Participant confidentiality was ensured throughout the study. Ethical approval with NHREC Approval Number: NHREC/17/03/2018/SHREC/2024/4838 was obtained from the Health Research Ethics Committee (HREC), Ministry of Health, Kano State.

Sample Collection

Participants were recruited for sample collection and were provided with detailed information on washing and drying techniques for the RMPs. They were instructed to follow specific washing protocols as highlighted in recommendations by UNICEF (2020). A total of 26 samples were collected and analysed from the participants of the study. Five (5) samples were collected from each participant. The samples collected included RMPs washed with water only (WWO), washed with Vivo detergent only (WD), washed with May bar soap only (WBS), washed with Vivo detergent and Dettol

disinfectant (WDD), and washed with Vivo detergent, Dettol disinfectant, and ironed (WDD-I) after drying in the sun. A sample of un-used RMP served as the control for the study. The samples (used RMPs) were retrieved from participants to analyze any microbial presence on the pads.

After cleaning, participants were instructed to handle the pads with clean hands and store them in sterile ziplock bags provided by the researchers. Participants washed the pads using the specified cleaning methods after their regular menstrual cycle. Researchers then retrieved the washed and dried pads for microbial analysis. This standardized collection protocol ensured consistency and reliability in sample handling, allowing for a thorough investigation of the effectiveness of different cleaning methods in reducing microbial contamination on RMPs.

Sample preparation

The samples were assigned unique identifiers and processed immediately to minimize microbial overgrowth. Each sample was aseptically removed from its sterile ziplock bag, immersed in 100 mL of buffered saline solution, and soaked for 2 minutes. The concentrated eluate was divided into several aliquots, and the analyses were performed using the membrane filtration technique (Briancesco *et al.*, 2018). Eight different groups of microorganisms/bacterial species were investigated, as highlighted by Briancesco *et al.* (2018). Thus, the membranes were incubated on various agarized media for the detection of the following microbial parameters:

The microbial analysis involved biochemical and molecular techniques to accurately identify the species present. The microbial analysis involved various organisms incubated under specific conditions to determine colony counts. Mesophilic bacteria were cultured on Plate Count Agar at 36°C for 72 hours, with total colonies counted. Fungi (molds and yeasts) were incubated on Sabouraud Dextrose Agar at 25°C for 7–10 days, and mold and yeast colonies were enumerated. Anaerobic bacteria were incubated on Plate Count Agar at 36°C in anaerobic conditions for 72 hours. Coliforms were identified on Chromogenic E. coli/Coliform (C-EC) medium at 36°C for 24 hours, with blue colonies counted. Escherichia coli colonies, both blue and fluorescent, were similarly counted using a Wood lamp. Staphylococcus spp. were identified on Baird Parker Agar at 36°C for 48 hours, with black colonies counted. Candida albicans was cultured on Biggy Agar at 36°C for 48 hours, with dark brown colonies enumerated. Finally, Pseudomonas aeruginosa was grown on Pseudomonas Agar at 36°C for 48 hours, and green-blue colonies were confirmed biochemically under a Wood lamp by identifying fluorescent and reddish-brown colonies.

Figure 1: Samples soaked in sterile peptone water

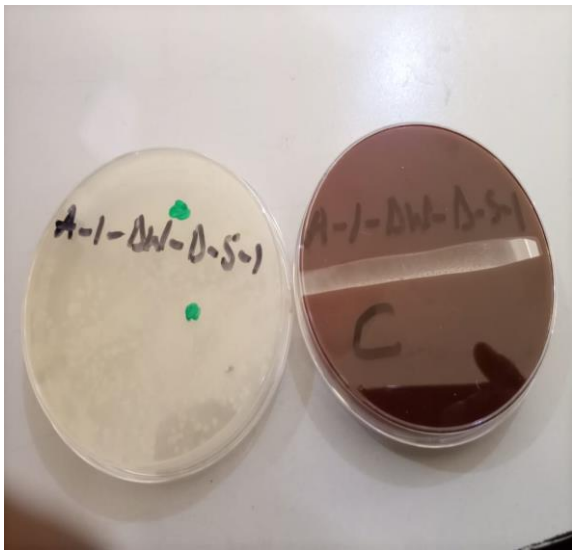

Figure 2: An overnight cultured plates (Nutrient and Heated blood agar)

Data Analysis

The number of colony-forming units (CFUs) on each plate was used to calculate each sample's total bacterial and fungal counts. Statistical analysis was performed to compare the microbial counts (and potentially the identified species) between RMPs cleaned with different methods.

RESULTS

The analysis reveals significant variation in microbial growth based on the cleaning method used. A large proportion of the pads (53.3%) showed no significant microbial growth when cleaned with effective methods such as detergent, disinfectant, and ironing, demonstrating the importance of proper cleaning techniques. Pads cleaned with water exhibited consistently high microbial counts, especially for mesophilic bacteria, often exceeding 100 CFU/RMP. Detergent cleaning alone resulted in a reduction in microbial load, though some samples still showed elevated counts. Bar soap yielded intermediate results, with microbial levels lower than water-only cleaning but still relatively high.

The most effective method for microbial reduction was the combination of detergent and disinfectant, which led to significant reductions in microbial growth, with most samples showing mesophilic bacterial counts below 50 CFU/RMP. Adding ironing to this cleaning method resulted in minimal to no microbial growth, with several samples showing no significant growth (NSG), indicating near-complete elimination of microbes. Molds, anaerobic bacteria, and *Staphylococcus spp.* were either undetectable or showed extremely low counts (<1 CFU/RMP) with this method. Unused pads exhibited low microbial counts, typically under 50 CFU/RMP, and pads sterilized by autoclaving showed no significant microbial growth, confirming the sterilization method's effectiveness in eliminating microbes.

Table 1: Result of Microbial Analysis of Reusable Menstrual Pads

PRODUCT CODE	MESOPHILIC BACTERIA CFU/ RMP	MOLDS CFU/ RMP	ANAEROBIC BACTERIA CFU/ RMP	*STAPHYLOCOCCUS* SPP. CFU/ RMP
A1-WWO	100	5	10	<1
S3-WWO	>100	10	<50	5
F4-WWO	>100	15	<50	5
M5-WWO	>100	10	<50	5
B2-WWO	>100	15	<50	5
A1-WD	<50	<1	<1	<1
S3-WD	<50	<1	<1	<1
F4-WD	>100	<1	50	<1
M5-WD	<100	<1	<50	<1
B2-WD	>100	<1	<50	<1
A1-WBS	<100	<1	<1	<1
B2-WBS	>100	5	<1	<1
S3-WBS	>50	<1	<1	<1
F4-WBS	>100	5	<1	<1
M5-WBS	100	<1	<1	<1
A1-WDD	<50	<1	<1	<1
B2-WDD	<1	<1	<1	<1
S3-WDD	<50	<1	<1	<1
F4-WDD	<100	<1	<1	<1
M5-WDD	<1	<1	<1	<1
A1-WDD-I	NG	<1	<1	<1
B2-WDD-I	NG	<1	<1	<1
S3-WDD-I	<1	<1	<1	<1
F4-WDD-I	<1	<1	<1	<1
M5-WDD-I	NG	<1	<1	<1
Un-used RMP	<50	<1	<1	<1
Un-used RMP sterilized by autoclave	NG	<1	<1	<1

CFU: Colony Forming Units NG: No Growth

Table 2: Chi-Square Test Results for All Dependent Variables

Dependent Variable	Test	Value	df	Asymptotic Significance (p-value)
Mesophilic Bacteria	Pearson Chi-Square	32.500	16	0.009
	Likelihood Ratio	35.467	16	0.003
	Linear-by-Linear Association	16.138	1	0.000
	N of Valid Cases			25
Molds	Pearson Chi-Square	26.667	12	0.009
	Likelihood Ratio	27.474	12	0.007
	Linear-by-Linear Association	10.446	1	0.001
	N of Valid Cases			25
Anaerobic Bacteria	Pearson Chi-Square	23.235	12	0.026
	Likelihood Ratio	26.881	12	0.008
	Linear-by-Linear Association	13.863	1	0.000
	N of Valid Cases			25
Staphylococcus spp.	Pearson Chi-Square	19.048	4	0.001
	Likelihood Ratio	16.979	4	0.002
	Linear-by-Linear Association	9.143	1	0.002
	N of Valid Cases			25

The Chi-Square test results show that the cleaning methods significantly impact mesophilic, molds, anaerobic, and Staphylococcus spp growth. The p-values for the different microbial types are as follows: for mesophilic bacteria, p = 0.009; for molds, p = 0.009; for anaerobic bacteria, p = 0.026; and for Staphylococcus spp., p = 0.001. These p-values indicate that the null hypotheses, which suggested no difference in microbial growth across cleaning methods, are rejected for all four microbial types because the p-values are less than the significance level of 0.05. Therefore, the cleaning method significantly influences microbial contamination levels, emphasizing the importance of selecting effective cleaning methods to reduce contamination and enhance the hygiene of reusable menstrual pads.

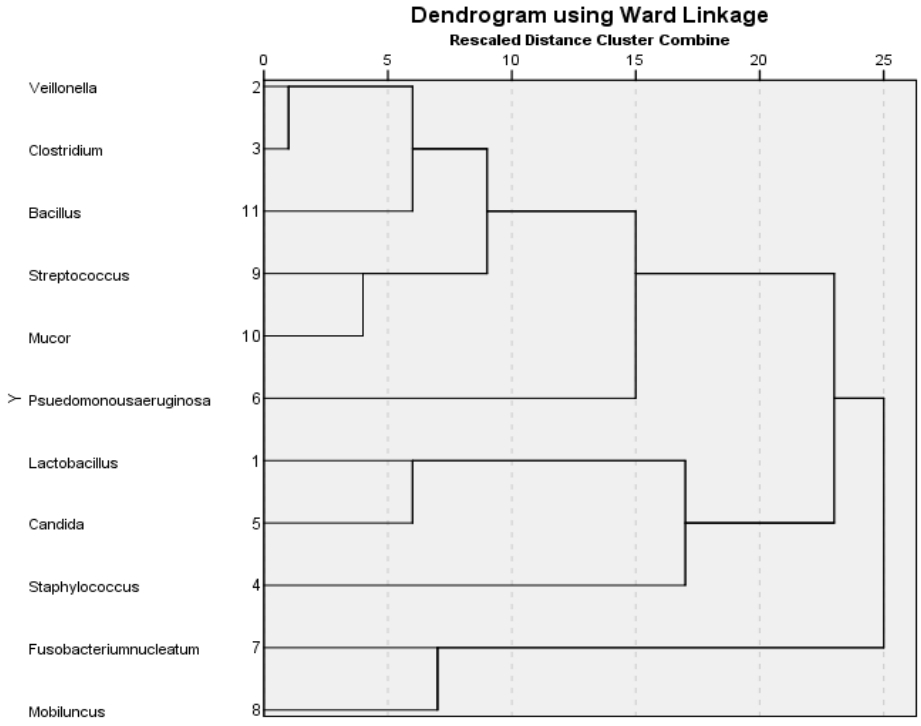

Figure 3: Dendrogram of Microbial Species Identified from the Analysed Reusable Menstrual Pads

The dendrogram illustrates the hierarchical clustering of microbial species identified from reusable menstrual pads based on their similarities and frequency of occurrence. The y-axis represents the rescaled distance between clusters, indicating how similar or dissimilar each cluster is. The first significant separation occurs with *Lactobacillus*, which forms its distinct cluster. Notably, *Lactobacillus* had the highest occurrence, appearing 6 times across the samples, highlighting its dominance in the microbial community. As the clustering progresses, *Veillonell*a and *Clostridium*, which appeared once each, form a group with other species, such as *Bacillus*, which also appeared once. These patterns suggest shared microbial characteristics among these species despite their lower frequency of occurrence.

The clustering also highlights species with moderate frequencies. For instance, *Staphylococcus* and *Candida*, each identified 5 times, merge in later stages of the dendrogram with species like *Pseudomonas aeruginosa*, which appeared 4 times, indicating potential co-occurrence or environmental similarities. At the same time, less frequent species such as *Fusobacterium nucleatum* and *Mobiluncus*, each identified 3 times, form a distinct cluster, suggesting a shared ecological niche on the reusable pads.

DISCUSSION

The effectiveness of different cleaning methods revealed significant variability in microbial presence. Pads washed with detergent and disinfectant and then ironed exhibited either minimal bacterial colonies or no microbial growth, demonstrating the superiority of this method in eliminating microorganisms. In contrast, other cleaning methods, such as washing with water only, detergent only, or bar soap only, showed varying degrees of microbial persistence. This result suggests that the combination of disinfectants and heat treatment (ironing) provides an effective strategy for reducing microbial contamination. The implications of these findings are crucial for promoting proper hygiene practices, especially in low-resource settings, to minimize health risks associated with reusable menstrual pads. Comparisons with previous studies, such as Veeh et al. (2003), further support the effectiveness of advanced cleaning and sterilization techniques in managing microbial contamination. However, studies such as Briancesco et al. (2018) highlight the limitations of relying solely on conventional cleaning methods, emphasizing the importance of educating users on optimal cleaning practices. These findings underline the need for further awareness and the standardization of hygiene protocols to ensure the safe use of RMPs.

Comparatively, studies examining microbial loads in other vaginal products, such as tampons, have shown varying results but emphasize the importance of adhering to stringent hygiene standards during production and use (Briancesco *et al.*, 2018; Veeh *et al.*, 2003). For instance, previous research has highlighted the presence of S. aureus biofilm on tampons, demonstrating the potential for biofilm formation and the importance of using advanced detection methods like fluorescent in situ hybridization (FISH) and PCR to accurately assess microbial presence (Veeh *et al.*, 2003). These insights highlight the need for continued vigilance in monitoring and improving hygiene practices associated with menstrual products to mitigate health risks effectively.

Species of fungi/mould species such as Candida and Aspergillus were implicated in opportunistic infections affecting the female genitals. Species of the Mucor genus are filamentous fungi found in the soil, plants, and decaying fruit. Usually, they do not cause diseases as they mostly do not survive temperatures as high as 37^0C. The microbial loads of the sample, as obtained by colony count, generally remained lower than the limit set by the FDA throughout the study and can pose little or no health risk to the product users(US Pharmacopoeia, 2016).

The Chi-Square analysis confirmed a significant relationship between cleaning methods and microbial contamination levels ($p < 0.05$ for mesophilic bacteria, molds, anaerobic bacteria, and Staphylococcus spp.). For instance, mesophilic bacterial contamination ($p = 0.009$) was most effectively reduced by the combination of detergent, disinfectant, and ironing, while molds ($p = 0.009$) and anaerobic bacteria ($p = 0.026$) showed similar patterns of reduction. These statistical results validate the critical role of proper cleaning practices in enhancing RMP hygiene and reducing health risks associated with microbial contamination.

The hierarchical clustering illustrated in the dendrogram further enriches the discussion by highlighting the ecological relationships and frequencies of the identified microorganisms. Lactobacillus was the most frequently occurring species (6 occurrences), highlighting its dominance in the microbial community.

In contrast, less frequent species like Clostridium and Veillonella (1 occurrence each) clustered with others such as Bacillus and Fusobacterium nucleatum, suggesting shared environmental niches. The co-occurrence of moderate-frequency species like Candida and Staphylococcus (5 occurrences each) with Pseudomonas aeruginosa (4 occurrences) underscores the potential for synergistic growth under suboptimal cleaning conditions.

The study's findings emphasize the critical need for education on effective RMP cleaning practices, especially in resource-limited settings. Integrating detergent, disinfectant, and ironing into routine cleaning protocols can mitigate microbial risks, promoting the safety and acceptability of RMPs as a sustainable menstrual health solution.

CONCLUSION

This study highlights the microbial contamination risks of reusable menstrual pads (RMPs) subjected to various cleaning methods, identifying diverse bacteria and yeast, including potentially pathogenic species such as Staphylococcus and Pseudomonas aeruginosa. Pads cleaned with water, detergent, or bar soap showed varying levels of microbial contamination, indicating these methods may be insufficient for optimal hygiene. In contrast, washing with detergent and disinfectant, especially when combined with ironing, significantly reduced microbial loads, demonstrating the effectiveness of this approach. These findings provide valuable insights into improving menstrual hygiene and safety, contributing to menstruating individuals' overall health and well-being.

Recommendations

The study recommends combining detergent, disinfectant, and ironing for effective microbial reduction on reusable menstrual pads (RMPs), as this method poses minimal health risks to users. However, sterilization through heat may offer the most reliable control of microbial growth. Future research should assess the long-term viability of bacteria on RMPs, evaluate various disinfectants, and refine ironing parameters to establish best practices for RMP hygiene and user safety.

Future studies should investigate the long-term viability of bacteria on RMPs, explore the efficacy of different disinfectants, and determine the optimal parameters for ironing as a potential additional disinfection step. By establishing best practices for RMP hygiene, we can help ensure the safety and well-being of users.

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Conflict of Interest

There is no conflict of interest in this project.

Author Contributions

AR contributed to the conceptualization, writing - original draft preparation, data analysis, and protocol development. UY contributed to methodology and protocol writing and served as the final reviewer and editor. FT participated in data collection, data analysis, and manuscript writing. SA and AT contributed to protocol development, project administration, and final draft review.

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