

Research Article

Evaluating the Microbiological Quality of Select Sanitary Pads Sold in Akure Metropolis, Ondo State, Nigeria

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Introduction

The sanitary pad, a fundamental element of women's menstrual health management, serves as an absorbent device used during menstruation and other situations necessitating blood absorption [1]. Beyond menstrual hygiene, sanitary pads find application post-vaginal surgeries, childbirth, and abortion [2]. From a historical and technological perspective, sanitary pads mark the inception of gynecological sanity and hygiene practices [3]. These products come in diverse variants, with winged disposable pads preferred for their added protection against leakage and enhanced stability [1]. Prior to disposable pads, reusable cloth pads made from various absorbent materials was the norm, underscoring the evolution of menstrual hygiene [4]. Sanitary pads encompass a range of types to cater to distinct needs. These include ultra-thin pads, regular pads, maxi/super pads, night pads, and maternity pads. Ultra-thin pads, characterized by compactness, deliver absorbency without bulkiness. Regular pads cater to the average flow, while maxi/super pads offer larger absorbent capacity for heavier menstruation. Night pads provide extended protection during recumbency, and maternity pads, slightly longer than maxi/super pads, manage post-childbirth, surgical, or abortion-related bleeding [5]. Sanitary pads are composed of three layers—the surface, absorbent, and underlying layers—each with specific considerations

Abstract

This study conducted microbiological evaluations of various brands of sanitary pads sold in Akure metropolis in Ondo State, Nigeria. The sanitary pads from different brands, including Always, Everyday, Ladycare, Ladychoice, Lovina, Rosemary, and Softcare, were collected and analyzed to determine the presence of microorganisms. Microorganisms were isolated from the sanitary pads using appropriate media such as Nutrient Agar and Potato Dextrose Agar, employing standard methods. The study revealed a diverse range of microorganisms, including bacteria such as *Bacillus cereus*, *Clostridium perfringens*, *Staphylococcus aureus*, *Veillonella parvula*, and *Lactobacillus antri*. Additionally, fungal species like *Rhizopus stolonifer*, *Aspergillus fumigatus*, *Aspergillus niger*, *Fusarium oxysporum*, and *Trichoderma sp.* were isolated from the sanitary pads. The presence of microorganisms on sanitary pads underscores their non-sterile nature. While some of the isolated microorganisms have the potential to cause infections, the vaginal pH and the presence of beneficial microorganisms like *Lactobacilli sp.* in the vagina may counteract the risk of infections from microorganisms present in sanitary pads. Thus, sanitary pads are considered safe for use. Nonetheless, maintaining a healthy lifestyle remains crucial.

Keywords: Sanitary pads; Microorganisms; Bacterial isolates; Fungal isolates; Vaginal pH; Women's health; Hygiene

in material choice and functional attributes. Surface layers necessitate rapid absorption to prevent skin wetness, while the absorbent middle layer requires effective absorption agents.

Microorganisms are ubiquitous and exist even on everyday objects, including sanitary pads, especially the widely used disposable varieties. Despite a common misconception that microbes are confined to clinical settings, they are present on various surfaces and materials frequently touched by hands, including sanitary pads [6]. This study illuminates the presence of microbial communities within sanitary pads, an aspect often overlooked but significant.

While sanitary pads are a staple in women's lives, the issue of their sterility has not garnered sufficient attention. These products, though clean, lack sterility and are not recognized as medical items, allowing manufacturers to abstain from listing their contents on packaging [7]. With compositions comprising wood fibers, cotton, rayon, polyester, polyacrylate, absorbency enhancers, chlorine compounds, and fragrances, sanitary pads could potentially harbor agents that cause infections [8]. Proximity to the skin, particularly the vulvar region, raises concerns about sanitary pads' involvement in various health issues, from vulvovaginitis to cancer [9].

Vulvar epithelial tissue, distinctive in structure and function, plays a role in safeguarding against harmful agents [10-12]. Research underscores the potential impact of synthetic underwear, tight pants, menstruation, and sanitary pad use on vulvar health, making the area prone to vulvovaginal diseases [13,14]. Notably, even individuals with normal and sensitive skin experience skin irritations due to the occlusion and humidity associated with sanitary pad use [2,15].

Against the backdrop of the non-sterile nature of sanitary pads and their potential implications on women's health, this study aims to quantitatively assess microbial presence in different brands of sanitary pads available in Akure, Ondo State, Nigeria. By establishing the sterility and safety of these products, the study aims to contribute to the understanding of microbial infections' epidemiology and pathogenesis, particularly concerning vulvovaginitis, contact dermatitis, and skin irritation, during and after menstruation. Given their close proximity to sensitive areas, sanitary pads must be devoid of microorganisms that pose health risks to users. The diverse microorganisms present in these products raise concerns about potential health implications. This study addresses this critical aspect of sanitary pad usage, contributing to the broader discourse on women's health and hygiene.

Materials and Methods

Sample Collection

Sanitary pad samples from brands including Always, Everyday, Lady Care, Lady Choice, Lovina, Softcare, and Rosemary were obtained from Oja Oba and NAO supermarket in Akure, Ondo State, Nigeria.

Materials Used

The laboratory equipment included an autoclave for sterilization, an incubator for controlled growth conditions, and a microscope for observations. Glassware such as McCartney Bottles, Test Tubes, Disposable Petri-Dishes, Conical Flasks, Beakers, and more were used. Sterilization techniques, a Bunsen burner, and culture media like Nutrient Agar, MacConkey Agar,

Blood Agar, and Potato Dextrose Agar were employed.

Laboratory Setup

To ensure the validity of our findings, we employed state-of-the-art laboratory equipment and techniques. Sterilization was executed using an autoclave, while incubation of cultures occurred in controlled conditions. Microscopic observations were made using advanced microscopy technology.

Sample Preparation

The collected sanitary pad samples were dissected into individual layers, and aliquots were soaked in sterile distilled water for microbial release.

Isolation and Characterization of Bacterial Isolates

The microbial samples were cultured on different media, including Nutrient Agar and Potato Dextrose Agar. The colonies were then characterized based on morphology, growth characteristics, and other features. Biochemical tests such as Catalase, Coagulase, Oxidase, Indole, Methyl Red, Urease, and Starch Hydrolysis were performed for identification according to the methods of Benson [16].

Isolation and Characterization of Fungal Isolates

Fungal isolates were identified based on colony characteristics and microscopic observations. Pure fungal cultures were obtained by transferring spores to Potato Dextrose Agar [17]

Results

In this study, a total of twenty-eight (28) samples were collected and analyzed. Analyses of seven (7) sanitary pads from different manufacturers were carried out. Three (3) samples were collected from each sanitary pad. The samples collected included the Surface Layer (SL), Absorbent Layer (AL), and Underlying area (UL) of each sanitary pad.

Bacteria Isolates

After the isolation of bacteria from the different sample ma-

Table 1: Morphological and Biochemical Characteristics of Fungi Isolates.

Organism	Morphological Characteristics	Gram Reaction	Catalase	Coagulase	Anaerobic	Citrate test	Starch hydrolysis	Lactose	Mannitol	Glucose	Sucrose	Maltose	Hemolysis	Spores
<i>Bacillus cereus</i>	White, Irregular Rough	+ <i>Bacillus</i>	+	-	+	+	+	-	-	+	+		+	+
<i>Staphylococcus aureus</i>	Cream smooth regular	+ <i>Cocci</i>	+	+	-	-	-	+	+	+	+	+	+	-
<i>Lactobacillus antri</i>	Cream Regular	+ <i>Bacillus</i>	+	NA	+	+	-	-	+	+	+	+	-	-
<i>Clostridium perfringens</i>	Cream smooth regular	+ <i>Bacillus</i>	-	NA	+	+	+	+	+	+	+	+	+	+
<i>Corynebacterium xerosis</i>	Cream wrinkled Irregular	+ <i>Bacillus</i>	+	NA	+	+	-	-	+	+	+	+	-	-
<i>Veillonella Parvula</i>	Cream smooth Rhizoid	- <i>Cocci</i>	-	NA	-	-	-	+	+	+	+	+	-	-
<i>Bacillus licheniformis</i>	Cream wrinkled irregular	+ <i>Bacillus</i>		NA	+	+	-	+		+	+	+	-	+

KEY: + = Positive, - = Negative, NA = NOT Applicable

materials used, the isolated bacteria were characterized based on their cellular, morphological, and biochemical properties. The details of the cellular, morphological, and biochemical characteristics of the bacterial isolates are shown in Table 1. These characteristics were employed for the identification of bacterial isolates. Biochemical tests, such as gram staining, catalase test, coagulase test, sugar fermentation, citrate test, and starch hydrolysis, were utilized for identification, with microorganisms testing positive or negative. The distribution of isolated bacteria on the examined sanitary pad surface layer is presented in Table 2. Seven different brands were analyzed for the presence of bacteria. Various bacterial species were isolated from four brands of sanitary pads analyzed, including Ladycare, Ladychoice, Rosemary, and Softcare. No bacteria were isolated from other brands of sanitary pads under analysis, such as Always, Everyday, and Lovina. Table 3 illustrates the distribution of bacterial isolates from the absorbent layer of the sanitary pads under analysis. Bacteria such as *Staphylococcus aureus*, *Veillonella parvula*, *Corynebacterium xerosis*, *Clostridium perfringens*, *Lactobacillus antri*, and *Bacillus licheniformis* were isolated from all the brands of sanitary pads.

Table 4 presents the distribution of bacterial isolates from the underlying layer of sanitary pads under analysis. Bacterial species such as *Clostridium perfringens*, *Corynebacterium*, *Veillonella parvula*, *Corynebacterium xerosis*, and *Staphylococcus aureus* were isolated from Everyday, Ladycare, Lovina, and Soft-

Table 2: Distribution of Bacteria Isolates in Surface Areas of Sanitary Pads.

Sanitary pads brand	Bacteria isolates
Always	No growth
Everyday	No growth
Ladycare	<i>Bacillus Cereus</i> <i>Bacillus licheniformis</i>
Lovina	No growth
Rosemary	<i>Lactobacillus antri</i>
Ladychoice	<i>Lactobacillus antri</i>
Softcare	<i>Staphylococcus aureus</i>

Table 3: Distribution of Bacteria Isolates In Absorbent of Sanitary Pads.

Sanitary pads brand	Bacteria isolates
Always	<i>Staphylococcus aureus</i>
Everyday	<i>Veillonella parvula</i>
Ladycare	<i>Corynebacterium xerosis</i> <i>Clostridium perfringens</i>
Lovina	<i>Bacillus licheniformis</i>
Rosemary	<i>Lactobacillus antri</i>
Ladycoice	<i>Corynebacterium xerosis</i> <i>Lactobacillus antri</i>
Softcare	<i>Veillonella parvula</i> <i>Staphylococcus aureus</i>

Table 4: Distribution of Bacteria Isolates in Underlying Areas of Sanitary Pads.

Sanitary pads brand	Bacteria isolates
Always	No growth
Everyday	<i>Corynebacterium xerosis</i>
Ladycare	<i>Veillonella parvula</i>
Lovina	<i>Clostridium perfringens</i>
Rosemary	No growth
Ladychoice	No growth
Softcare	<i>Staphylococcus aureus</i>

care sanitary pads, respectively. No bacteria were isolated from Always, Ladychoice, and Rosemary sanitary pads, respectively.

A total of five fungi were isolated from all the samples. The details of the colonial and morphological characteristics of the fungal isolates are illustrated in Table 5. Fungal species isolated from the sanitary pads include *Rhizopus stolonifer*, *Aspergillus fumigatus*, *Aspergillus niger*, *Fusarium oxysporum*, and *Trichoderma sp.* These fungal isolates were obtained from the different sanitary pads analyzed. The frequency of occurrence of the fungal isolates differs, with *Rhizopus stolonifer* and *Aspergillus fumigatus* having a high occurrence, while *Aspergillus niger* and *Fusarium oxysporum* have a low occurrence frequency. *Fusarium oxysporum* occurred once in the absorbent layer of Always, while *Trichoderma sp.* exhibited an average occurrence frequency. The distribution of fungal isolates in the surface layer of the sanitary pads analyzed is presented in Table 6. Six out of the seven sanitary pads analyzed were positive for fungal growth, with Always sanitary pads being negative for fungal growth.

The distribution of fungal isolates in the absorbent layer of the sanitary pads is shown in Table 7. No fungi were isolated from Rosemary sanitary pads, while fungi were isolated from the other brands analyzed. Table 8 shows the contribution of fungal isolates in the underlying area of the analyzed sanitary pads. The Ladycare sanitary pad underlying layer was negative for fungal growth, while *Rhizopus stolonifer* and *Aspergillus fumigatus* occurred in all the remaining six brands of sanitary pads analyzed.

Discussion

The presence of microorganisms in sanitary pads indicates that these products are not sterile and can disrupt the equilibrium of the vulvar microbiota [10,18]. Menstrual cycles render women more susceptible to vulvovaginal infections due to shifts in vulvar pH and microbiota [19,20]. This microbiota usually prevents the growth of microorganisms capable of causing vaginitis [21-23]. Beyond microbiota changes potentially triggered by sanitary pad use, alterations in environmental factors like humidity, pH, and temperature can occur, fostering the proliferation of exogenous bacteria and fungi [10]. Vaginal pH, a gauge of acidity, typically ranges from 3.8 to 5.0 in healthy women. Menstruation elevates vaginal pH due to the alkaline nature of blood [24]. Although these pH shifts can lead to vaginal issues, many women's bodies can adapt to them [25]. Sanitary napkins, including pads and tampons, further impact vaginal pH by absorbing menstrual fluid along with both endogenous and exogenous bacteria. They disrupt endogenous bacteria that typically maintain pH balance, potentially providing a surface for the growth of exogenous bacteria [25].

This study involved the isolation of diverse microorganisms from various sanitary pad brands. Isolated fungi included *Rhizopus stolonifer*, *Aspergillus fumigatus*, *Aspergillus niger*, *Fusarium oxysporum*, and *Trichoderma sp.* Fungi flourish in slightly acidic environments with a pH around 5 and low moisture levels. While *Aspergillus fumigatus* and *Aspergillus niger* are known to cause infections like allergic reactions and lung infections in various organs [26], *Aspergillus* species are seldom implicated in opportunistic fungal infections affecting the female genital [27]. *Fusarium oxysporum*, linked to infections such as fungal keratitis and onychomycosis, tends to affect immunocompromised individuals, especially in cutaneous and subcutaneous infections [28,29]. Although *Trichoderma sp.* and *Rhizopus stolonifer* can be pathogenic, they are rarely associated with causing vaginal

Table 5: Morphological Characterization of Fungi Isolates.

Colour of colony	Characterization of fungi growth and microscopic view	Isolate
Brownish-black	Large and radiate conidial heads and is biserriate. Conidiospores and smooth walled	<i>Aspergillus niger</i>
White spore with orange background	Conidiospore are short, single; microconidia are fusiform, slightly curved. Septate mycelium bearing crescent conidia on the conidiophores	<i>Fusarium oxysporum</i>
White then brownish as it age	A dense cottony growth characterized by the presence of stolon and pigmented rhizoids, the formation of sporangiospores, non-septate chlamdospores	<i>Rhizopus stolonifer</i>
Smoky gray green with slight yellow reverse	Septate branched mycelium, grey green conidia, ascospores present, conidiophores are smooth walled and terminated in a domed shaped vesicle	<i>Aspergillus fumigatus</i>
Army green	Branched conidiospores, main branched produce lateral side branches that may not be branched	<i>Trichoderma sp</i>

Table 6: Frequency of Fungal Species on Surface Layer.

Brand of sanitary pad	Fungal species	Occurrence frequency
Always	No growth	
Everyday	<i>Rhizopus stolonifer</i>	3
	<i>Aspergillus fumigatus</i>	3
Ladycare	<i>Rhizopus stolonifer</i>	1
	<i>Aspergillus fumigatus</i>	2
Lovina	<i>Aspergillus fumigatus</i>	1
Rosemary	<i>Rhizopus stolonifer</i>	1
	<i>Aspergillus niger</i>	6
	<i>Aspergillus fumigatus</i>	1
Ladychoice	<i>Rhizopus stolonifer</i>	5
	<i>Aspergillus fumigatus</i>	4
Softcare	<i>Rhizopus stolonifer</i>	3
	<i>Aspergillus fumigatus</i>	2

Table 7: Frequency of Fungal Species on Absorbent Layer.

Brand of sanitary pad	Fungal species	Occurrence frequency
Always	<i>Fusarium oxysporum</i>	1
	<i>Rhizopus stolonifer</i>	1
Everyday	<i>Rhizopus stolonifer</i>	3
	<i>Aspergillus fumigatus</i>	3
Ladycare	<i>Aspergillus fumigatus</i>	1
Lovina	<i>Aspergillus niger</i>	1
	<i>Trichoderma sp</i>	6
Rosemary	No growth	
Ladychoice	<i>Rhizopus stolonifer</i>	5
	<i>Aspergillus fumigatus</i>	7
Softcare	<i>Aspergillus fumigatus</i>	5

Table 8: Frequency of Fungal Species on Underlying Layer.

Brand of sanitary pad	Fungal species	Occurrence frequency
Always	<i>Rhizopus stolonifer</i>	1
Everyday	<i>Rhizopus stolonifer</i>	2
	<i>Aspergillus fumigatus</i>	1
Ladycare	No growth	
Lovina	No growth	
Rosemary	<i>Aspergillus fumigatus</i>	2
Ladychoice	<i>Aspergillus niger</i>	2
	<i>Aspergillus fumigatus</i>	2
Softcare	<i>Rhizopus stolonifera</i>	2
	<i>Aspergillus fumigatus</i>	3

infections [28]. Despite their potential to grow in the vulvar region, the isolated fungi's presence on sanitary pads may not hold significant medical relevance for vulvar infections.

Isolated bacteria comprised *Bacillus cereus*, *Clostridium perfringens*, *Staphylococcus aureus*, *Veillonella parvula*, and *Lactobacillus antri*. *Veillonella parvula* is part of the normal oral flora [31,32] and has been found in women with bacterial vaginosis [33]. *Staphylococcus aureus* is linked to various vaginal infections. While *Clostridium perfringens* rarely causes infections in female genital organs and can induce colpitis (inflammation of

the vagina), such conditions often have a dramatic course [30]. Some isolated bacteria in this study are capable of causing vaginal infections. However, clinical infections require alterations in host resistance or bacterial load [34,35]. Establishing vulvar infections by these bacteria hinges on microbiota shifts that facilitate bacterial growth from sanitary pads. Aside from microbiota changes, conditions triggered by sanitary pads during menstruation, such as heightened humidity, increased pH, and anaerobic conditions, can foster the proliferation and activities of these bacteria.

Conclusion

The presence of microorganisms in sanitary pads underscores their ubiquity and potential for growth in various environments, including sanitary pads. These microorganisms can proliferate and potentially lead to infections around the vulva, particularly during and after menstruation when changes in the vulva's environment occur. However, the mere existence of unknown, exogenous, and potentially pathogenic species does not automatically equate to disease, especially when the disease is defined in terms of noticeable symptoms.

The assortment of microorganisms found in sanitary pads, including some opportunistic pathogens, may not necessarily result in vaginal infections. This is because the vaginal microflora plays a crucial role in preventing vulva colonization by maintaining a lower vaginal pH, rendering many of these microorganisms unable to thrive. Therefore, while sanitary pads may not be entirely sterile, they can still be considered safe for appropriate use.

Recommendations

Based on the findings of this study, it is recommended that women avoid wearing sanitary pads continuously for extended periods of time (several hours). Prolonged use can lead to an increased multiplication of microorganisms, particularly bacteria, which may disturb the natural balance of the vaginal ecosystem, potentially leading to infections. Additionally, women should adopt and maintain healthy lifestyles to promote a balanced vaginal ecosystem. This proactive approach will help prevent the colonization of the vagina by any microorganisms that might be present in the sanitary pads being used.

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