

Microbiological Characterization of Sanitary Pad Sold in Port Harcourt Metropolis

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ABSTRACT

This study investigated the microbial loads and diversity of bacteria and fungi associated with unused sanitary pads sold in selected markets and pharmacies in Port Harcourt Metropolis, Rivers State, Nigeria. A total of 216 sanitary pads were obtained from six retail outlets over three sampling periods. Samples were analysed using standard cultural methods to enumerate total heterotrophic bacteria, total coliforms, total staphylococci, and fungi. Representative isolates were characterized using morphological and biochemical methods for presumptive identification. Microbial counts varied among the sampled products and retail locations. Total heterotrophic bacterial counts (THB) ranged from $(3.0 \times 10^4 \text{ CFU/g})$ in Lady Care and Lily Pad samples from Obio/Akpor Market to $(125.6 \times 10^4 \text{ CFU/g})$ in Molped samples from Ikwerre Pharmacy. Total coliform counts (TCC) ranged from undetectable levels to $(8.3 \times 10^4 \text{ CFU/g})$ in Dry Love samples from Ikwerre Market. Total staphylococcal counts (TSC) ranged from undetectable levels to $(149.6 \times 10^3 \text{ CFU/g})$ in Molped samples from Port Harcourt Pharmacy, while total fungal counts (TF) ranged from undetectable levels to $(26.7 \times 10^3 \text{ CFU/g})$ in Lily Pad samples from Obio/Akpor Pharmacy. Bacterial isolated included *Bacillus* sp., *Staphylococcus* sp., *Escherichia coli*, *Enterobacter* sp., *Klebsiella* sp., *Serratia* sp., *Lactobacillus* sp., *Micrococcus* sp., *Citrobacter* sp., and *Pseudomonas* sp. Fungal isolates comprised *Aspergillus* sp., *Candida* sp., and *Penicillium* sp. *Bacillus* sp., *Staphylococcus* sp., *E. coli*, and *Enterobacter* sp. were recovered across all six sampling locations. The findings indicate that the sampled sanitary pads were not microbiologically sterile and contained varying populations of culturable bacteria and fungi. However, the recovery of these microorganisms does not establish that the products cause infections or are unsafe for use. Confirmatory microbial identification and further investigations into the clinical relevance of the recovered isolates are needed to clarify their potential implications for the health of users.

Keywords: Sanitary pads, bacterial isolates, fungal isolates, microbiological quality, Port Harcourt Metropolis.

Introduction

Sanitary pads are widely used menstrual hygiene products designed to absorb menstrual fluid and support personal hygiene. Their use forms an important component of menstrual hygiene management, which encompasses the materials, facilities, and practices required to manage menstruation safely and with dignity (Dasgupta & Sarkar, 2008; Sommer et al., 2015).

However, the microbiological quality of these products may be influenced by manufacturing processes, packaging, transportation, storage, retail handling, and environmental conditions.

Since sanitary pads remain in contact with the external genital region during use, evaluating their microbial content is relevant to understanding their quality and potential health implications (Farage & Maibach, 2007). Microorganisms are widely distributed in the environment and can persist on frequently handled surfaces and consumer products. Studies have demonstrated that everyday surfaces may serve as reservoirs for microorganisms, including potentially pathogenic bacteria (Bures et al., 2009). Although commercially available sanitary pads are not necessarily sterile, their microbial quality remains an important consideration in product safety.

A study by Aboh et al. (2021) specifically investigated consumer perceptions and the microbiological quality of sanitary pads sold in Nigeria's Federal Capital Territory, highlighting the relevance of examining these products within local retail environments. In addition, variations in product composition and exposure to environmental conditions may influence their quality. Research on menstrual products has also examined their chemical composition and potential exposure implications, although these concerns are distinct from microbial contamination (Park et al., 2019; Upson et al., 2022). The isolation and identification of bacteria and fungi from sanitary pads can provide useful information about the microbial diversity associated with these products. Some recovered organisms may be environmental contaminants, while others may include opportunistic or potentially pathogenic microorganisms. Nevertheless, the detection of a microorganism on a sanitary pad does not, by itself, establish that the product causes infection. The significance of contamination depends on factors such as organism identity, abundance, pathogenic characteristics, exposure conditions, and host susceptibility. Research on vulvovaginal health has demonstrated the importance of interactions among the vaginal microbiota, mucosal defences, and host immunity in maintaining microbial balance and preventing infection (Balakrishnan et al., 2022; Kalia et al., 2020). Studies have also investigated associations between sanitary pad or clothing use and vulvovaginitis, although such associations do not independently establish that contaminated products cause these conditions (Bardin et al., 2013). Bacterial persistence on surfaces may also be influenced by biofilm formation. Biofilms are structured microbial communities attached to surfaces and enclosed within an extracellular matrix, which can promote microbial persistence under certain environmental conditions (Hall-Stoodley et al., 2004). The ability of bacteria to attach to surfaces and form biofilms is therefore relevant to understanding their survival and potential persistence on consumer products. In addition, fungal contamination warrants attention because filamentous fungi and other fungal groups may occur in different environmental settings. Egbuta et al. (2017) discussed potential health risks associated with exposure to filamentous fungi, although the recovery of fungi from a product alone does not demonstrate a health hazard.

Despite the widespread use of sanitary pads, information on the microbiological characteristics of products available in different Nigerian retail environments remains limited. Differences in storage, handling, product turnover, and environmental exposure may contribute to variations in the microbial populations recovered from products sold in markets and pharmacies. Local investigations are therefore necessary to document bacterial and fungal contamination and establish baseline information on the microbiological quality of commercially available sanitary pads.

This study assessed the microbiological quality of sanitary pads sold in selected markets and pharmacies in Port Harcourt Metropolis, Rivers State, Nigeria. It involved the enumeration, isolation, and presumptive identification of bacterial and fungal contaminants using cultural and phenotypic methods. The findings provide information on the microbial loads and diversity associated with the sampled products and contributes to the evidence base for evaluating the microbiological quality of sanitary pads in the study area.

Materials and Methods

Study Area

The study was conducted in Port Harcourt metropolis and adjoining areas in Rivers State, Nigeria. Unused disposable sanitary pads were purchased from six retail outlets comprising Oil Mill Market and a pharmacy in Obio/Akpor Local Government Area (LGA), Mile 1 Market and a pharmacy in Port Harcourt LGA, and Igwuruta Market and a pharmacy in Ikwerre LGA.

Study Design and Sample Collection

A laboratory-based descriptive study was conducted to characterize the culturable bacterial and fungal populations associated with unused disposable sanitary pads sold in Port Harcourt metropolis. Twelve (12) pads were obtained from each of the six selected retail outlets during each sampling period over three sampling periods making a total of 216 sanitary pads. Purposive sampling was used to select brands of sanitary pad from markets and pharmacies.

Only unused, sealed, unexpired disposable sanitary pads with intact packaging were included. Expired products and those with damaged packaging were excluded. The collected samples were labelled appropriately and transported to the Microbiology Laboratory of Rivers State University, Port Harcourt, for microbiological analysis.

Sample Preparation and Microbiological Analysis

The external surfaces of the collected sanitary pads were swabbed for microbial isolation. In addition, 10 g of each pad sample was weighed aseptically and homogenized in 90 mL of sterile normal saline (Aboh et al., 2021). The resulting suspension was subjected to serial dilution for microbial enumeration. A separate control was prepared by wrapping sanitary pad samples in aluminium foil and autoclaving them at 121°C and 15 psi for 15 minutes. The sterilized samples were subsequently cultured to assess the effectiveness of the sterilization procedure.

Enumeration of Total Heterotrophic Bacterial Counts

An aliquot of 0.1 mL from the (10^{-4}) dilution was inoculated onto sterile, solidified Nutrient Agar (NA) using the spread-plate technique. The plates were incubated at 37°C for 24 hours. Following incubation, visible colonies were counted, and bacterial counts were expressed as colony-forming units per gram (CFU/g) of sanitary pad sample, where applicable. Discrete colonies were selected aseptically and subcultured repeatedly to obtain pure cultures. The purified isolates were preserved in 10% (v/v) glycerol suspension for subsequent characterization and identification (Williams and Penu, 2018).

Enumeration of Coliforms and Staphylococci

An aliquot of 0.1 mL from the (10^{-3}) dilution was spread-plated onto Eosin Methylene Blue (EMB) agar and Mannitol Salt Agar (MSA) for the recovery and enumeration of presumptive coliform bacteria and staphylococci, respectively. The inoculated plates were incubated at 37°C for 24–48 hours. Following incubation, colonies were counted and recorded. Representative colonies were subcultured to obtain pure isolates for further characterization.

Enumeration of Fungal Counts

An aliquot of 0.1 mL from the (10^{-3}) dilution was spread-plated onto Sabouraud Dextrose Agar (SDA) supplemented with tetracycline to inhibit bacterial growth. The plates were incubated at 28°C for 5–7 days. Fungal colonies were counted, and representative colonies were subcultured onto SDA slants for purification and preservation pending identification. Fungal counts were expressed as CFU/g of sample, where applicable.

Phenotypic Characterization and Identification of Bacterial Isolates

Bacterial isolates were characterized based on colonial morphology, cellular morphology, Gram reaction, motility and biochemical characteristics. Colony characteristics, including size, shape, colour, texture, margin and elevation, were examined visually. Gram staining was performed to determine the Gram reaction, cellular shape and arrangement of the isolates (Aleruchi et al., 2026).

Motility was assessed using semisolid motility medium. Biochemical tests included catalase, oxidase, indole, citrate utilization, methyl red, Voges–Proskauer and carbohydrate fermentation tests. Coagulase testing was also performed where appropriate for presumptive staphylococcal isolates. The results of the morphological and biochemical tests were compared with standard identification criteria in Bergey’s Manual of Determinative Bacteriology (Holt et al., 1994) to achieve presumptive identification of the bacterial isolates.

Morphological Identification of Fungal Isolates

Fungal isolates were characterized based on their colonial and microscopic features. Colonial characteristics, including colour, texture, growth pattern, surface appearance and pigmentation, were examined visually. Microscopic examination was performed using the lactophenol cotton blue (LPCB) staining technique. A small portion of each fungal colony was transferred to a clean glass slide containing a drop of LPCB stain and gently teased apart using a sterile inoculating needle to disperse the fungal structures. A coverslip was carefully placed over the preparation, avoiding air bubbles.

The slide was examined under the $\times 10$ objective to locate fungal structures and the $\times 40$ objective for detailed observation. Microscopic features, including hyphal septation, branching, conidiophores, conidia, sporangia and the arrangement and shape of spores, were recorded. The observed colonial and microscopic characteristics were compared with standard descriptions in Larone's Medically Important Fungi: A Guide to Identification (Walsh et al., 2018) for presumptive identification of the fungal isolates.

Statistical Analysis

Microbiological data were organized and analysed using the Statistical Package for the Social Sciences (SPSS). Bacterial and fungal counts were summarized using appropriate descriptive statistics. Differences between groups were assessed using analysis of variance (ANOVA), where applicable, with statistical significance set at ($p < 0.05$).

Results

The total heterotrophic bacterial load of sanitary pads varied across brands, sales outlets and local government areas (Table 1). In Obio/Akpor, significant differences were observed among market-sourced brands ($P < 0.001$), but not pharmacy-sourced brands ($P = 0.074$). In Port Harcourt, bacterial loads differed significantly among brands purchased from both markets and pharmacies ($P < 0.001$). Similarly, significant differences were observed among market-sourced ($P = 0.003$) and pharmacy-sourced ($P < 0.001$) products in Ikwerre. Molped recorded the highest mean bacterial load among pharmacy-sourced products in Obio/Akpor (88.7×10^4 CFU/g), Port Harcourt (90.0×10^4 CFU/g) and Ikwerre (125.6×10^4 CFU/g). These findings demonstrate variation in the heterotrophic bacterial loads of sanitary pads across brands and sampling locations.

Table 1: Total Heterotrophic Bacterial Load (CFU/g) of Sanitary Pad from Markets and Pharmacies

Sample	Oil Mill Obio/Akpor LGA		Mile 1 Port Harcourt LGA		Igwuruta Ikwerre LGA	
	Market ($\times 10^4$)	Pharmacy ($\times 10^4$)	Market ($\times 10^4$)	Pharmacy ($\times 10^4$)	Market ($\times 10^5$)	Pharmacy ($\times 10^4$)
A	47.0 $\pm$ 22.5 ^b	10.6 $\pm$ 9.8 ^a	10.6 $\pm$ 9.8 ^a	9.7 $\pm$ 4.1 ^a	0.40 $\pm$ 0.0 ^a	7.0 $\pm$ 0.0 ^a
Ag	9.7 $\pm$ 5.8 ^a	3.3 $\pm$ 1.2 ^a	9.7 $\pm$ 4.2 ^a	3.3 $\pm$ 1.2 ^a	10.2 $\pm$ 9.3 ^{cd}	4.3 $\pm$ 0.6 ^a
B	4.0 $\pm$ 0.0 ^a	4.3 $\pm$ 0.6 ^a	79.0 $\pm$ 25.5 ^b	7.7 $\pm$ 1.2 ^a	1.2 $\pm$ 0.5 ^{ab}	38.0 $\pm$ 3.4 ^{ab}
DL	8.3 $\pm$ 4.5 ^a	6.0 $\pm$ 0.0 ^a	8.3 $\pm$ 1.5 ^a	9.0 $\pm$ 1.7 ^a	12.7 $\pm$ 7.5 ^d	6.0 $\pm$ 0.0 ^a
Dp	4.3 $\pm$ 0.6 ^a	6.0 $\pm$ 0.0 ^a	9.7 $\pm$ 2.3 ^a	8.3 $\pm$ 1.5 ^a	4.7 $\pm$ 1.6 ^{abc}	8.3 $\pm$ 1.5 ^a
LC	3.0 $\pm$ 1.7 ^a	8.0 $\pm$ 0.0 ^a	9.7 $\pm$ 4.2 ^a	75.3 $\pm$ 52.3 ^{bc}	1.3 $\pm$ 0.4 ^{ab}	75.3 $\pm$ 5.2 ^{abc}
LP	3.0 $\pm$ 0.0 ^a	4.0 $\pm$ 0.0 ^a	78.3 $\pm$ 30.6 ^b	3.3 $\pm$ 1.2 ^a	1.7 $\pm$ 0.3 ^{abc}	3.0 $\pm$ 0.0 ^a
M	7.0 $\pm$ 0.0 ^a	88.7 $\pm$ 9.8 ^b	31.3 $\pm$ 8.1 ^a	90.0 $\pm$ 3.9 ^c	8.3 $\pm$ 4.7 ^{bcd}	125.6 $\pm$ 8.7 ^c
MG	5.0 $\pm$ 0.0 ^a	7.3 $\pm$ 4.5 ^a	21.3 $\pm$ 8.1 ^a	37.3 $\pm$ 16.2 ^{ab}	0.30 $\pm$ 0.2 ^a	4.3 $\pm$ 0.6 ^a
S	4.3 $\pm$ 0.6 ^a	33.3 $\pm$ 31.7 ^a	9.7 $\pm$ 2.3 ^a	81.3 $\pm$ 51.4 ^c	5.4 $\pm$ 2.8 ^{abc}	113.0 $\pm$ 77.3 ^c
Vir	3.0 $\pm$ 0.0 ^a	9.7 $\pm$ 2.3 ^a	8.5 $\pm$ 2.1 ^a	9.7 $\pm$ 2.3 ^a	0.33 $\pm$ 0.11 ^a	3.0 $\pm$ 0.0 ^a
WG	4.0 $\pm$ 0.0 ^a	4.3 $\pm$ 0.6 ^a	20.0 $\pm$ 3.5 ^a	3.3 $\pm$ 1.2 ^a	0.83 $\pm$ 0.4 ^a	81.7 $\pm$ 25.1 ^{bc}
P-value	<0.001	0.074	<0.001	<0.001	0.003	<0.001

*Means with similar superscript down the group showed no significant differences ($P > 0.05$)

Key: A- Always, AG- Angel, DL- Dry love, DP- Dele pad, LC-Lady care, LP-lily pad, M-Molped, MG- My Girl, S-soft care, Vir-Virony, WG-Wondergirl.

The total coliform load of sanitary pads varied across brands, sales outlets and local government areas (Table 2). In Obio/Akpor, significant differences were observed among brands purchased from both markets

($P < 0.001$) and pharmacies ($P = 0.002$). In Port Harcourt, significant differences occurred among brands from markets and pharmacies ($P < 0.001$) for both).

In Ikwerre, differences were not statistically significant among market-sourced products ($P=0.070$), but were significant among pharmacy-sourced products ($P<0.001$). Molped recorded the highest total coliform load among market-sourced products in Obio/Akpor (8.0×10^3 CFU/g), while Soft Care had the highest value among pharmacy-sourced products

in the same LGA (18.0×10^3 CFU/g). In Ikwerre, Molped from pharmacies had the highest reported load (19.0×10^3 CFU/g). These findings indicate variation in total coliform loads among sanitary pad brands and sampling locations, although the magnitude and statistical significance of these differences varied across groups.

Table 2: Total Coliform Load (CFU/g) of Sanitary Pad from Markets and Pharmacies

Sample	Oil Mill Obio/Akpor LGA		Mile 1 Port Harcourt LGA		Igwuruta Ikwerre LGA	
	Market ($\times 10^3$)	Pharmacy ($\times 10^3$)	Market ($\times 10^3$)	Pharmacy ($\times 10^3$)	Market ($\times 10^4$)	Pharmacy ($\times 10^3$)
A	0.0 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a	1.0 $\pm$ 0.0 ^{ab}	0.0 $\pm$ 0.0 ^a	8.0 $\pm$ 0.0 ^c
Ag	1.0 $\pm$ 0.0 ^c	4.0 $\pm$ 0.0 ^a	4.3 $\pm$ 2.9 ^{abcd}	4.0 $\pm$ 0.0 ^c	4.6 $\pm$ 1.1 ^{ab}	1.0 $\pm$ 0.0 ^a
B	0.0 $\pm$ 0.0 ^a	8.0 $\pm$ 0.0 ^a	7.7 $\pm$ 4.0 ^{cd}	7.0 $\pm$ 3.5 ^d	5.9 $\pm$ 4.7 ^{ab}	9.0 $\pm$ 0.0 ^c
DL	0.0 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a	3.0 $\pm$ 1.7 ^{ab}	0 $\pm$ 0.0 ^a	8.3 $\pm$ 1.0 ^b	0.0 $\pm$ 0.0 ^a
Dp	1.0 $\pm$ 0.0 ^c	0.0 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a	3.0 $\pm$ 1.7 ^{bc}	4.1 $\pm$ 0.4 ^{ab}	3.0 $\pm$ 1.7 ^b
LC	0.0 $\pm$ 0.0 ^a	3.0 $\pm$ 0.0 ^a	1.0 $\pm$ 0.0 ^a	3.0 $\pm$ 1.7 ^{bc}	0.0 $\pm$ 0.0 ^a	3.0 $\pm$ 1.7 ^b
LP	0.0 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a	8.0 $\pm$ 3.5 ^d	4.0 $\pm$ 0.0 ^c	0.20 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a
M	8.0 $\pm$ 0.0 ^d	2.0 $\pm$ 0.0 ^a	0 $\pm$ 0.0 ^a	3.0 $\pm$ 1.7 ^{bc}	0.20 $\pm$ 0.0 ^a	19.0 $\pm$ 0.0 ^d
MG	0.66 $\pm$ 0.57 ^b	0.0 $\pm$ 0.0 ^a	6.3 $\pm$ 2.9 ^{bcd}	3.3 $\pm$ 1.2 ^{bc}	0.0 $\pm$ 0.0 ^a	1.0 $\pm$ 0.0 ^a
S	0.0 $\pm$ 0.0 ^a	18.0 $\pm$ 15.6 ^b	0.0 $\pm$ 0.0 ^a	1.3 $\pm$ 0.58 ^a	2.5 $\pm$ 0.0 ^{ab}	4.0 $\pm$ 0.0 ^b
Vir	0.0 $\pm$ 0.0 ^a	3.0 $\pm$ 1.7 ^a	3.5 $\pm$ 1.7 ^{abc}	0 $\pm$ 0.0 ^a	0.40 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a
WG	1.0 $\pm$ 0.0 ^c	0.0 $\pm$ 0.0 ^a	4.3 $\pm$ 2.9 ^{abcd}	4.0 $\pm$ 0.0 ^c	0.0 $\pm$ 0.0 ^a	4.0 $\pm$ 0.0 ^b
P-value	<0.001	0.002	<0.001	<0.001	0.07	<0.001

*Means with similar superscript down the group showed no significant differences ($P>0.05$)

Key: A- Always, AG- Angel, DL- Dry love, DP- Dele pad, LC-Lady care, LP-lily pad, M-Molped, MG- My Girl, S-soft care, Vir-Virony, WG-Wondergirl

The total staphylococcal load of sanitary pads differed significantly among brands across all three local government areas (Table 3). In Obio/Akpor, My Girl recorded the highest load among market-sourced products (13.0×10^3 CFU/g), while Lady Care had the highest load among pharmacy-sourced products (22.0×10^3 CFU/g). In Port Harcourt, My Girl recorded the highest market load (11.7×10^3 CFU/g), whereas Molped had the highest pharmacy load (149.6×10^3 CFU/g). In Ikwerre, Dry Love recorded the highest market load (12.8×10^4 CFU/g), while Baby (B) recorded the highest pharmacy load (99.7×10^3 CFU/g), followed by Molped (93.3×10^3 CFU/g). Significant differences were observed among brands in all market and pharmacy groups ($P \leq 0.035$), indicating variation in staphylococcal loads by brand and sampling location.

Table 4 shows that total fungal load of sanitary pads varied across brands, sales outlets and LGAs. In Obio/Akpor, significant differences were observed among market-sourced products ($P=0.049$), but not pharmacy-sourced products ($P=0.270$). Angel and My Girl recorded the highest market fungal loads (16.3×10^3 CFU/g each), while Lily Pad had the highest pharmacy load (26.7×10^3 CFU/g). In Port Harcourt, fungal loads differed significantly among brands purchased from both markets and pharmacies ($P<0.001$) for both, with Wondergirl recording the highest market load (4.7×10^3 CFU/g) and Baby the highest pharmacy load (5.0×10^3 CFU/g). In Ikwerre, significant differences were also observed among market-sourced ($P=0.002$) and pharmacy-sourced products ($P<0.001$). Wondergirl had the highest market fungal load (7.3×10^3 CFU/g), while Soft Care recorded the highest pharmacy load (5.0×10^3 CFU/g).

Table 3: Total Staphylococcal Load (CFU/g) of Sanitary Pad from Markets and Pharmacies

Sample	Oil Mill Obio/Akpor LGA		Mile 1 Port Harcourt LGA		Igwuruta Ikwerre LGA	
	Market ($\times 10^3$)	Pharmacy ($\times 10^3$)	Market ($\times 10^3$)	Pharmacy ($\times 10^3$)	Market ($\times 10^4$)	Pharmacy ($\times 10^3$)
A	0.0 $\pm$ 0.0 ^a	16.3 $\pm$ 3.7 ^{bc}	10.3 $\pm$ 9.6 ^{bc}	4.7 $\pm$ 3.1 ^a	0.0 $\pm$ 0.0 ^a	1.0 $\pm$ 0.0 ^a
Ag	1.0 $\pm$ 0.0 ^a	3.7 $\pm$ 2.3 ^{ab}	3.0 $\pm$ 1.7 ^a	3.7 $\pm$ 2.3 ^a	2.1 $\pm$ 2.0 ^a	3.3 $\pm$ 2.8 ^a
B	3.3 $\pm$ 2.8 ^a	0.0 $\pm$ 0.0 ^a	0.66 $\pm$ 0.58 ^a	12.0 $\pm$ 8.7 ^a	0.0 $\pm$ 0.0 ^a	99.7 $\pm$ 98.6 ^b
DL	0.66 $\pm$ 0.57 ^a	2.3 $\pm$ 1.2 ^a	0 $\pm$ 0.0 ^a	0 $\pm$ 0.0 ^a	12.8 $\pm$ 5.6 ^b	2.3 $\pm$ 1.1 ^a
Dp	3.3 $\pm$ 2.8 ^a	3.0 $\pm$ 0.0 ^{ab}	3.7 $\pm$ 2.3 ^a	0 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a
LC	3.0 $\pm$ 0.0 ^a	22.0 $\pm$ 3.1 ^c	4.7 $\pm$ 3.1 ^{ab}	2.0 $\pm$ 1.7 ^a	0.0 $\pm$ 0.0 ^a	2.0 $\pm$ 1.7 ^a
LP	0.66 $\pm$ 0.57 ^a	1.3 $\pm$ 1.1 ^a	3.0 $\pm$ 1.7 ^a	3.7 $\pm$ 2.3 ^a	2.4 $\pm$ 1.1 ^a	0.67 $\pm$ 0.6 ^a
M	0.0 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a	0 $\pm$ 0.0 ^a	149.6 $\pm$ 59.5 ^b	5.4 $\pm$ 0.7 ^a	93.3 $\pm$ 5.2 ^b
MG	13.0 $\pm$ 11.5 ^b	1.3 $\pm$ 1.1 ^a	11.7 $\pm$ 4.0 ^c	1.7 $\pm$ 0.58 ^a	0.30 $\pm$ 0.0 ^a	3.3 $\pm$ 2.8 ^a
S	3.3 $\pm$ 2.8 ^a	8.3 $\pm$ 8.0 ^{ab}	1.3 $\pm$ 1.2 ^a	4.3 $\pm$ 2.3 ^a	2.6 $\pm$ 1.2 ^a	74.3 $\pm$ 67.8 ^b
Vir	0.66 $\pm$ 5.7 ^a	0.0 $\pm$ 0.0 ^a	0 $\pm$ 0.0 ^a	1.3 $\pm$ 1.2 ^a	3.7 $\pm$ 2.3 ^a	0.66 $\pm$ 0.5 ^a
WG	2.0 $\pm$ 0.0 ^a	3.3 $\pm$ 2.9 ^a	3.7 $\pm$ 2.3 ^a	3.7 $\pm$ 2.3 ^a	0.066 $\pm$ 0.01 ^a	8.0 $\pm$ 0.0 ^a
P-value	0.016	0.035	0.002	<0.001	<0.01	0.007

*Means with similar superscript down the group showed no significant differences (P>0.05)

Key: A- Always, AG- Angel, DL- Dry love, DP- Dele pad, LC-Lady care, LP-lily pad, M-Molped, MG- My Girl, S-soft care, Vir-Virony, WG-Wondergirl

Table 4: Total Fungal Load (CFU/g) of Sanitary Pad from Markets and Pharmacies

Sample	Oil Mill Obio/Akpor LGA		Mile 1 Port Harcourt LGA		Igwuruta Ikwerre LGA	
	Market ($\times 10^3$)	Pharmacy ($\times 10^3$)	Market ($\times 10^3$)	Pharmacy ($\times 10^3$)	Market ($\times 10^3$)	Pharmacy ($\times 10^3$)
A	9.3 $\pm$ 7.5 ^{ab}	0.0 $\pm$ 0.0 ^a	0 $\pm$ 0.0 ^a	0 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a	4.0 $\pm$ 0.0 ^d
Ag	16.3 $\pm$ 6.3 ^b	1.0 $\pm$ 0.0 ^a	1.0 $\pm$ 0.0	1.0 $\pm$ 0.0 ^b	1.0 $\pm$ 0.0 ^{ab}	1.0 $\pm$ 0.0 ^a
B	4.0 $\pm$ 0.0 ^{ab}	2.0 $\pm$ 0.0 ^a	2.7 $\pm$ 0.58 ^d	5.0 $\pm$ 0.0 ^f	0.0 $\pm$ 0.0 ^a	3.0 $\pm$ 0.0 ^c
DL	8.0 $\pm$ 6.9 ^{ab}	2.0 $\pm$ 0.0 ^a	2.0 $\pm$ 0.0 ^c	1.0 $\pm$ 0.0 ^b	4.3 $\pm$ 1.2 ^{bc}	2.0 $\pm$ 0.0 ^b
Dp	1.0 $\pm$ 0.0 ^a	1.0 $\pm$ 0.0 ^a	2.7 $\pm$ 0.58 ^d	2.0 $\pm$ 0.0 ^c	1.0 $\pm$ 0.0 ^{ab}	2.0 $\pm$ 0.0 ^b
LC	3.0 $\pm$ 0.0 ^a	3.0 $\pm$ 0.0 ^a	0 $\pm$ 0.0 ^a	0 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a	1.0 $\pm$ 0.0 ^a
LP	4.0 $\pm$ 0.0 ^{ab}	26.7 $\pm$ 3.7 ^b	1.0 $\pm$ 0.0 ^b	1.0 $\pm$ 0.0 ^b	2.0 $\pm$ 0.0 ^{ab}	4.0 $\pm$ 0.0 ^d
M	2.0 $\pm$ 0.0 ^a	1.0 $\pm$ 0.0 ^a	1.0 $\pm$ 0.0 ^b	3.3 $\pm$ 0.58 ^e	3.0 $\pm$ 0.0 ^{ab}	1.3 $\pm$ 0.6 ^a
MG	16.3 $\pm$ 1.8 ^b	3.0 $\pm$ 0.0 ^a	1.0 $\pm$ 0.0 ^b	2.7 $\pm$ 0.58 ^d	3.0 $\pm$ 0.0 ^{ab}	1.3 $\pm$ 0.6 ^a
S	1.0 $\pm$ 0.0 ^a	2.0 $\pm$ 0.0 ^a	1.0 $\pm$ 0.0 ^b	2.3 $\pm$ 0.58 ^{cd}	1.0 $\pm$ 0.0 ^{ab}	5.0 $\pm$ 0.0 ^e
Vir	4.0 $\pm$ 0.0 ^{ab}	2.7 $\pm$ 0.6 ^a	2.0 $\pm$ 0.0 ^c	1.0 $\pm$ 0.0 ^b	1.0 $\pm$ 0.0 ^{ab}	4.0 $\pm$ 0.0 ^d
WG	0.66 $\pm$ 5.7 ^a	1.0 $\pm$ 0.0 ^a	4.7 $\pm$ 0.58 ^e	1.0 $\pm$ 0.0 ^b	7.3 $\pm$ 6.4 ^c	3.0 $\pm$ 0.0 ^c
P-value	0.049	0.270	<0.001	<0.001	0.002	<0.001

*Means with similar superscript down the group showed no significant differences (P>0.05)

Key: A- Always, AG- Angel, DL- Dry love, DP- Dele pad, LC-Lady care, LP-lily pad, M-Molped, MG- My Girl, S-soft care, Vir-Virony, WG-Wondergirl

The distribution of presumptive bacterial isolates across the six sampling groups is presented in Table 5. *Bacillus* sp., *Staphylococcus* sp., *Escherichia coli* and *Enterobacter* sp. were reported in all sampling locations, indicating their widespread occurrence among the sampled sanitary pads. *Klebsiella* sp. was detected in five of the six groups.

While *Serratia* sp. occurred in three groups. *Lactobacillus* sp. and *Micrococcus* sp. were each detected in four groups, whereas *Citrobacter* sp. was reported only in Oil Mill Market. *Pseudomonas* sp. was detected exclusively in Igwuruta Market.

The distribution of presumptive fungal isolates across the six sampling groups is presented in Table 6. *Aspergillus* sp., *Candida* sp. and *Penicillium* sp. were detected in all sampling locations, irrespective of whether the sanitary pads were purchased from markets or pharmacies. These findings indicate the widespread occurrence of the three fungal genera among the sampled sanitary pads across Mile 1, Oil Mill and Igwuruta. However, the presence records alone do not establish differences in their prevalence or abundance among locations. Identification was presumptive, based on colonial and microscopic characteristics, and was not confirmed by molecular methods.

Table 5: Distribution of presumptive bacterial isolates across sampling locations

Bacterial isolate	Mile 1 Port Harcourt LGA		Oil Mill Obio/Akpor LGA		Igwuruta Ikwerre LGA	
	Market	Pharmacy	Market	Pharmacy	Market	Pharmacy
<i>Bacillus</i> sp.	+	+	+	+	+	+
<i>Staphylococcus</i> sp.	+	+	+	+	+	+
<i>Escherichia coli</i>	+	+	+	+	+	+
<i>Enterobacter</i> sp.	+	+	+	+	+	+
<i>Klebsiella</i> sp.	+	—	+	+	+	+
<i>Serratia</i> sp.	+	—	—	—	+	+
<i>Lactobacillus</i> sp.	—	+	—	—	+	+
<i>Micrococcus</i> sp.	—	—	+	+	+	+
<i>Citrobacter</i> sp.	—	—	+	—	—	—
<i>Pseudomonas</i> sp.	—	—	—	—	+	—

Key: + = reported as present; — = not reported in the supplied distribution records. Market and pharmacy samples are treated as separate sampling groups.

Table 6: Distribution of presumptive fungal isolates across sampling locations

Fungal isolate	Oil Mill Obio/Akpor LGA		Mile 1 Port Harcourt LGA		Igwuruta Ikwerre LGA	
	Market	Pharmacy	Market	Pharmacy	Market	Pharmacy
<i>Aspergillus</i> sp.	+	+	+	+	+	+
<i>Candida</i> sp.	+	+	+	+	+	+
<i>Penicillium</i> sp.	+	+	+	+	+	+

Key: + = reported as present; — = not reported in the supplied distribution records. Market and pharmacy samples are treated as separate sampling groups.

Discussion

The recovery of culturable bacteria and fungi from unused sanitary pads indicates variation in the microbiological quality of the products examined. Although sanitary pads are intended for menstrual hygiene, their microbial quality may be influenced by manufacturing hygiene, packaging, storage and handling before purchase. Aboh et al. (2021), in their assessment of sanitary pads sold in Nigeria, provide a relevant local context for evaluating the microbiological quality of these products. However, the present findings do not establish when or where the recovered organisms were introduced. The results should therefore be interpreted as evidence of microbial recovery from unused products rather than proof of contamination at a specific stage of production or distribution.

The variation in total heterotrophic bacterial counts suggests that microbial quality differed among brands and sampling groups. Such differences may reflect variation in production processes, raw materials, packaging integrity or storage conditions, although these factors were not directly examined. The importance of maintaining hygienic menstrual management conditions is discussed by Sommer et al. (2015), while Chen et al. (2017) consider the relationship between intimate hygiene practices and vulvovaginal health. These studies provide a broader context for product hygiene, but they do not establish that bacterial recovery from sanitary pads causes genital disease. Total heterotrophic counts are indicators of culturable microbial burden and should not be interpreted as direct measures of pathogenicity or infection risk (Benson, 2002). The recovery of coliforms provides an additional indication of differences in the microbiological quality of the products. Coliforms are used in hygiene assessment, but their presence alone does not confirm faecal contamination or establish the presence of a specific enteric pathogen. The recovery of presumptive *Escherichia coli* and other enteric-associated bacteria warrants careful interpretation because phenotypic identification may not reliably establish species identity or pathogenic characteristics. The biological significance of enteric bacteria depends on the organism involved, its virulence properties, the extent of exposure and the susceptibility of the exposed host (Bergstrom et al., 2012).

Confirmatory identification and further characterisation would therefore be required before drawing conclusions about the potential health implications of these isolates. The recovery of presumptive staphylococci is also noteworthy because staphylococci occur on human skin and in the environment. Their presence may be consistent with several possible routes of introduction, including contact during handling, but the study did not investigate contamination pathways. Research on bacterial biofilms has demonstrated that microorganisms can persist in diverse environments and on surfaces, although this evidence does not establish biofilm formation on the sanitary pads examined in this study (Hall-Stoodley et al., 2004). Consequently, the recovery of staphylococci should be discussed in terms of product-associated bacterial occurrence rather than assumed pathogenicity. Species-level identification and relevant virulence testing would be needed to establish whether the isolates have particular clinical significance. The fungal findings indicate that culturable fungi were also associated with the products examined. Fungal recovery may be influenced by environmental exposure, packaging and storage conditions, but these potential explanations were not directly tested. Egbuta et al. (2017) discuss health risks associated with exposure to filamentous fungi, while Bandres et al. (2023) describe the clinical relevance of *Aspergillus fumigatus*. These sources establish that some fungi can be medically important, but their relevance to the present study is limited unless the recovered isolates were identified to an appropriate taxonomic level. The detection of a fungal genus does not demonstrate that a pathogenic species or mycotoxin was present, nor does it establish that the product would cause infection. The distribution of bacterial taxa across the sampled groups suggests that some presumptive organisms were widely recovered, whereas others occurred in fewer groups. This pattern may reflect differences in microbial occurrence, the characteristics of the products sampled or the capacity of the culture methods to recover particular organisms. The presence of *Bacillus*, *Staphylococcus*, *E. coli* and *Enterobacter* across the sampling groups does not, however, establish a common contamination source. Similarly, the more restricted recovery of other taxa should not automatically be interpreted as evidence of better product hygiene in the groups where they were not detected.

Culture-based non-detection does not necessarily mean that an organism was completely absent, as recovery depends on sampling and laboratory conditions. The widespread recovery of *Aspergillus*, *Candida* and *Penicillium* further demonstrates that fungal isolates were not confined to one sampling group. Their potential significance differs by species and context. In particular, *Candida* is relevant to vulvovaginal health, but vulvovaginal candidiasis is a multifactorial condition involving interactions among the host, vaginal mucosa, immunity and the resident microbiota (Balakrishnan et al., 2022; Kalia et al., 2020). The recovery of *Candida* from a sanitary pad does not demonstrate that the organism originated from a user, disrupted the vaginal microbiota or caused candidiasis. Such conclusions would require clinical evidence and investigation of the relationship between product exposure and health outcomes. The same caution applies to the other fungal genera recovered. The findings should also be considered in relation to the scope of the study. Microbial recovery from unused sanitary pads provides information about culturable organisms associated with the products at the time of examination; it does not directly measure exposure during use or establish adverse health effects. The study did not determine the source of the organisms, confirm all identifications molecularly, assess virulence determinants or establish a relationship between product use and infection. Consequently, the results cannot establish that the examined products caused genital infections or other health conditions. Future investigations should include confirmatory identification, relevant virulence characterisation and assessment of manufacturing, packaging and storage conditions. Where health risks are being evaluated, these investigations should be complemented by appropriate exposure and clinical data.

Conclusion

This study demonstrated the recovery of culturable bacteria and fungi from unused sanitary pads sold in selected markets and pharmacies in Port Harcourt Metropolis, Rivers State. Variation in microbial counts and the distribution of presumptive bacterial and fungal taxa indicate differences in the microbiological quality of the sampled products. These findings provide baseline information but do not establish the pathogenicity of the recovered organisms or demonstrate a risk of infection among users.

The findings support the importance of quality control and hygienic practices during sanitary-pad manufacture, packaging, storage and distribution. Further studies incorporating confirmatory microbial identification and assessment of clinically relevant characteristics are needed to clarify the implications of these findings for product quality and consumer health.

References

- Aboh, M.I., Ekpo, M., Khalid-Salako, F.A. & Oladosu, P.O. (2021). Consumers' perception on safety and microbiological assessment of sanitary pads sold in the Federal Capital Territory (FCT), Nigeria. *Journal of Phytomedicine and Therapeutics*, 20(1), 598–614.
- Aleruchi, O., Ogbuleka, N.A.C. and Onwukwe, N.A. (2026). Bacteriological assessment and antibiotic susceptibility patterns of bacteria isolated from hospital door handles in Port Harcourt, Nigeria. *Microbiology Research International*, 14(2), 29-38.
- Balakrishnan, S.N., Yamang, H., Lorenz, M.C., Chew, S.Y. & Than, L.T.L. (2022). Role of vaginal mucosa, host immunity and microbiota in vulvovaginal candidiasis. *Pathogens*. 11(6), 618.
- Bandres, M.V., Modi, P. & Sharma, S. (2023). *Aspergillus fumigatus*. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; Available from: <https://www.ncbi.nlm.nih.gov/books/NBK482464/>
- Bardin, M.G., Giraldo, P.C., Pinto, C.L.B., Piassaroli, V.B., Gomes do Amaral, R.L., & Polpetta, N. (2013). Association of sanitary pads and clothing with vulvovaginitis. *DST—Jornal Brasileiro de Doenças Sexualmente Transmissíveis*, 25, 123–127.
- Benson, H.J. (2002). Bacterial population counts. In: Microbiological Applications: Laboratory Manual in General Microbiology. 8th ed. New York: McGraw-Hill. p. 87.
- Bergstrom, K.S., Sham, H.P., Zarepour, M., & Vallance, B.A. (2012). Innate host responses to enteric bacterial pathogens: a balancing act between resistance and tolerance. *Cellular Microbiology*. 14, 475–484.

- Bures, S., Fishbain, J.T., Uyehara, C.F., Parker, J.M. & Berg, B.W. (2009) Computer keyboards and faucet handles as reservoirs of nosocomial pathogens in the intensive care unit. *American Journal of Infection Control*, 28(6), 465–471.
- Chen, Y., Bruning, E., Rubino, J. & Eder, S.E. (2017). Role of female intimate hygiene in vulvovaginal health: global hygiene practices and product usage. *Women's Health*, 13(3), 58–67.
- Dasgupta, A. & Sarkar, M. (2008). Menstrual hygiene: how hygienic is the adolescent girl? *Indian Journal of Community Medicine*, 33(2), 77–80.
- Egbuta, M.A., Mwanza, M. & Babalola, O.O. (2017). Health risks associated with exposure to filamentous fungi. *International Journal of Environmental Research and Public Health*. 14(7), 719.
- Farage, M.A. & Maibach, H. (2007). Cumulative skin irritation test of sanitary pads in sensitive skin and normal skin population. *Cutaneous and Ocular Toxicology*, 26, 37–43.
- Hall-Stoodley, L., Costerton, J.W. & Stoodley, P. (2004). Bacterial biofilms: from the natural environment to infectious diseases. *Nature Reviews Microbiology*, 2, 95–108.
- Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T. & Williams, S.T. (1994). *Bergey's Manual of Determinative Bacteriology*, 12-18.
- Kalia, N., Singh, J. & Kaur, M. (2020). Microbiota in vaginal health and pathogenesis of recurrent vulvovaginal infections: a critical review. *Annals of Clinical Microbiology and Antimicrobials*, 19, 5.
- Park, C.J., Barakat, R., Ulanov, A., Li, Z., Lin, P.C. & Chiu, K. (2019). Sanitary pads and diapers contain higher phthalate contents than those in common commercial plastic products. *Reproductive Toxicology*, 84, 114–121.
- Sommer, M., Hirsch, J.S, Nathanson, C., & Parker, R.G. (2015). Comfortably, safely, and without shame: defining menstrual hygiene management as a public health issue. *American Journal of Public Health*, 105(7), 1302–1311.
- Upton, K., Shearston, J.A., & Kioumourtzoglou, M.A. (2022). Menstrual products as a source of environmental chemical exposure: a review from the epidemiologic perspective. *Current Environmental Health Reports*. 9, 38–52.
- Walsh, T. J., Hayden, R. T. & Larone, D. H. (2018). Larone's medically important fungi: A guide to identification (6th ed.). ASM Press.
- Williams, J. O. & Penu, B. S. (2018). Bioremediation Potential of *Aspergillus clavatus* and *Pichia spp.* on oil Spill Dispersant Polluted Wetland. *Journal of Advances in Biology and Biotechnology*, 19(4), 1-14.