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Bacteriological analysis of the sanitary pads available in the local markets of Surat, Gujarat, India

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Abstract - Sanitary pads are widely used menstrual hygiene products that come into direct contact with the skin and mucosal surfaces for extended periods, making their microbial safety a critical public health concern. This study focuses on the bacteriological analysis of commercially available sanitary pads to assess their bacterial contamination levels and ensure user safety. Samples from various brands, including those sold as loose pickings, were subjected to standard bacteriological procedures, including the isolation and identification of probable contaminants, as well as the determination of antibiotic resistance in the isolates. The results indicated that *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter* spp., *Klebsiella* spp., *Pseudomonas aeruginosa*, and *Bacillus spp.* were the probable bacterial isolated from different sanitary pads. The probable isolates exhibited antibiotic resistance; 3 strains of *S. aureus* were found to be resistant to tetracycline, penicillin and combine resistant to penicillin and streptomycin, 2 strains of *E.coli* one resistant to ampicillin another resistant to both tetracycline and chloramphenicol, *Enterobacter spp*. strain was found resistant to both tetracycline and Penicillin G, while one strain each of *B. cereus* and *Klebsiella spp*. were resistant to ampicillin. The present study provides insight into which types of sanitary pads should be purchased, as well as recommendations for their storage at home. The presence of antibiotic-resistant bacterial isolates in sanitary pads raises concerns about their prolonged use.

***Keywords*:** Sanitary pads, bacterial contaminants, antibiotic resistant isolates, menstruation, microbial quality control, sterility

1. INTRODUCTION *(Size 11, Times New roman)*

Sanitary pads—also referred to as sanitary napkins or menstrual pads—are slim, absorbent products made from specialized materials. Their primary purpose is to absorb menstrual fluid and offer comfort and protection during menstruation. The U.S. Food and Drug Administration (FDA) classifies sanitary pads as low-risk medical devices and recommends pre-clinical microbiological testing to ensure they are free of harmful microorganisms, such as *Staphylococcus aureus*, which could compromise vaginal health. Given the ubiquity of microorganisms, improper storage of sanitary pads may lead to contamination (Aboh *et al*., 2021).

Studies in India have revealed that in many areas, the use of sanitary pads remains low. Furthermore, women with reproductive tract infections are often found to have poorer menstrual hygiene practices (Dhingra *et al*., 2009; Kansal *et al*., 2016; Das *et al.,* 2015). The preference for sanitary protection materials is influenced by personal choice, cultural acceptability, economic status, and local availability (Kaur et al., 2018). In addition to basic sanitation facilities, the provision of soap and menstrual absorbents is essential for proper menstrual hygiene management (MHM). The choice of absorbents often differs between rural and urban populations.

However, the sterility of these products remains a topic of concern. To ensure product quality, the Bureau of Indian Standards (BIS) has issued specifications under IS 5405 (1980), which manufacturers are expected to follow. While large-scale manufacturers are typically certified under international quality standards and subject to hygiene testing, smaller manufacturers are not always regulated. In states such as Rajasthan and Uttar Pradesh, government agencies procure pads from small-scale units for distribution in public institutions. User feedback has indicated that these products are often of inferior quality, with poor absorbency and visible contamination within the packaging (Development Solutions & WSSCC, 2018).

Therefore, the present study aims to isolate and identify potential bacteria from commercially available sanitary pads, including branded products such as Whisper, Stayfree, and She, as well as unbranded pads sold in loose packaging in local markets of Surat City. Additionally, the study seeks to determine the presence of antibiotic-resistant bacterial isolates, given their implications for women's reproductive health and menstrual hygiene management.

2. MATERIALS AND METHODS

Analysis of bacterial contamination in different sanitary pads was carried out using following steps;

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2.1 Sample collection: Unused sanitary pads from various branded and branded companies were purchased from medical stores, the mega marts and in the loose packs from the local vendors as well.

2.2 Processing of samples: Whole piece of sanitary pad was used for isolation of bacterial contaminants. By removing the cover of the sanitary pad, it was chopped off in small pieces with help of sterile scissors, forces and a tray for keeping pad in to it under sterile condition (between two burners).

2.3 Enrichment of samples in liquid media: The chopped pieces of sanitary pad were transferred to the 250 ml flask containing enrichment broth; Nutrient and peptone broth (60 ml) and incubated at 37 ºC for 24h (Figure 1).

Figure 1: Chopped pieces of sanitary pads in the enrichment broth

2.4 Isolation of bacterial contaminants: After 24 hrs. of incubation the enrichment broths were observed for the turbidity. Broth with the turbidity was further proceeded to gram staining followed by the streaking on the solid media; Nutrient agar plate, MacConkey agar plate (for Gram negative) and Mannitol Salt Agar (MSA) (for gram positive) incubated for 24 hrs. at 37ºC. The broth without turbidity and the solid media with no bacterial colonies after 24 hrs. were further incubated up to 72 hrs.

2.5 Identification of isolated bacteria: As per the standard procedure the single colony of the probable isolated bacteria was partially identified from their grams reaction, colony morphology on different solid media and biochemical characteristics **(**Patel. R & Patel. K, 2016).

2.6 Antibiotic Susceptibility Testing (AST) by Kirby Bauer Disc Diffusion Method: The antibiotic susceptibility test was performed using standard protocol in Muller-Hinton agar with 0.1 ml of selected probable isolates against commercially available antibiotic discs viz., Vancomycin (VA-30 µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Gentamycin (10µg), Levoflaxain (5µg), Penicillin G (10µg), Streptomycin (10µg) and Tetracycline (TE-30) and they incubated at 37 °C for 24 hrs **(**Patel. R & Patel. K, 2016).

3. RESUTS AND DISCUSSION

In this study, a total of thirty (30) samples were collected and analyzed. Sanitary pads manufactured by different companies were analyzed, namely: Sofy (2 pads), Whisper (2 pads), Airiz (2 pads), Niine Naturally Soft (2 pads), Stay Sure (2 pads), Soft & Secure (2 pads), Extra Sure (2 pads), Ultra Soft (2 pads), Super Soft Ultra-Secure (2 pads), Stayfree (2 pads), and sanitary pads in loose packs with no brand name (10 pads).

3.1 Isolation and Identification of the probable bacterial isolates.

Each sanitary pad sample was purchased from a different lot-numbered packet, except for those in loose packing. After the isolation of bacteria from the various sample materials, the isolated bacteria were characterized based on their cellular, morphological, and biochemical properties. Commonly found probable bacterial isolates were *S. aureus, Bacillus spp., E. coli, Enterobacter spp., Klebsiella spp., and Pseudomonas aeruginosa,* whereas many isolates remained unidentified. Table 1 presents details of some morphological and biochemical characteristics. These isolates are considered probable only, as limited biochemical and morphological tests were performed.

Table 1: Identification of the probable isolates from the sanitary pads

Probable Bacterial isolate	Morphological characteristics on solid media	Gram's reaction	Positive Biochemical tests	Negative Biochemical tests	Positive tests for Sugar fermentation
S. aureus	On MSA-Cream smooth regular, yellow colonies with a yellow zone	Gram positive cocci	Catalase, Citrate, Gelatine hydrolysis, MR, VP, Urease	Coagulase, Indole, H2S, Oxidase	Glucose, Fructose, Lactose, Maltose & Mannitol
Bacillus cereus	On NA-Large, irregular and	Gram positive roads	Catalase, Citrate, VP, Casein hydrolysis,	Indole, MR, Gelatine	Fructose, Glucose, Maltose

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	granular colonies		Esculin hydrolysis	hydrolysis, Oxidase	
Bacillus subtilis	On NA- white, irregular, rough, opaque colonies	Gram positive rods	Catalase, Citrate, Gelatine Hydrolysis, VP	Indole, MR, Urease	Arabinose, Galactose, Glucose, Maltose, Mannitol
E. coli	On EMB- small colonies with greenish metallic sheen	Gram negative short rods	Catalase, Indole, MR, TSI (Acid/Acid, Gas +),	Gelatine Hydrolysis, Citrate, VP, Urease	Glucose, Xylsoe, Lactose, Maltose & Mannitol (Acid+Gas)
Enterobacter spp.	On EMB- mucoid, dark centred colonies, slightly violet or pinkish coloration	Gram negative short rods	Catalase, Citrate, VP, TSI (Acid/Acid, Gas +)	Indole, MR, Gelatine hydrolysis, Urease	Glucose, Fructose, Lactose, Maltose & Mannitol (Acid+Gas)
Klebsiella spp.	On MaCConkey- Large, mucoid, sticky and slimy colonies	Gram negative short rods	Similar as *Enterobacter spp.* the difference was in the colony morphology		
Pseudomonas aeruginosa	On NA- large, opaque, flat, irregular colonies with green coloration	Gram negative short rods	Catalase, Citrate, Gelatine hydrolysis, Oxidase, TSI (Alkaline/ Alkaline)	Coagulase, Indole, MR, VP, Urease	Fructose, Mannitol

The presence of various bacterial contaminants may be attributed to poor sterility of the pad, substandard quality of raw materials used, inadequate packaging, and, most importantly, improper storage of these items before and after sale. Sanitary pads are generally stored in bathrooms alongside toiletries. Evidence of coliforms such as *E. coli* and *Enterobacter* spp. indicates that these sanitary pads may have been poorly manufactured and stored, raising concerns about female vaginal health. Table 2 presents the details of the probable bacterial isolates identified from the analysed sanitary pads.

Table 2: List of probable bacterial isolates found from the analyzed sanitary pads

Sample no.	Sanitary pad/company name	No. of isolates found	Probable identified bacteria
S-1	Sofy	2	*S. aureus*
S-2		2	*S. aureus*
S-3	Whisper	2	Unidentified gram positive *S. aureus*
S-4		0	No growth
S-5	Airiz	1	*Bacillus cereus.*
S-6		0	No growth
S-7	Niine Naturally soft	1	Unidentified gram positive bacteria
S-8		1	Unidentified gram negative bacteria
S-9	Stay Sure	1	*Bacillus spp.*
S-10		1	*Bacillus spp.*
S-11	Soft & Secure	1	*S. aureus*
S-12		0	No growth
S-13	Extra Sure	1	Unidentified gram positive
S-14		1	*Bacillus spp.*
S-15	Ultra Soft	1	*Bacillus spp.*
S-16		0	No growth
S-17	Super soft Ultra Secure	1	Unidentified gram positive
S-18		0	No growth
S-19	Stay free	2	*E. coli,* Unidentified gram positive
S-20		0	No growth
S-21	Sanitary pads in loose pickings without company name	1	*S. aureus, Klebsiella spp.*
S-22		1	*S. aureus, E. coli, Enterobacter spp.*
S-23		1	*Bacillus spp.*
S-24		2	*S. aureus*, *Enterobacter spp.*
S-25		1	Unidentified gram positive
S-26		1	*S. aureus, Bacillus spp.*
S-27		1	*E. coli, Bacillus spp.*
S-28		1	*Enterobacter spp, Bacillus subtilis*.
S-29		1	*Pseudomonas aeruginosa, Bacillus spp.*
S-30		1	*Bacillus spp.*

This study involved the isolation of diverse microorganisms from various sanitary pad brands. A total of 34 isolates were identified at least to the genus level, whereas 7 isolates remained unidentified. Additionally, 5 pad samples, specifically from Whisper, Airiz, Soft & Secure, Ultra Soft, and Stayfree—showed no bacterial growth even after 72 hours of incubation. A study

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from Nigeria reported a diverse range of microorganisms, including bacterial species such as *Bacillus cereus*, *Clostridium perfringens*, *Staphylococcus aureus*, *Veillonella parvula*, and *Lactobacillus antri*, as well as fungal species such as *Rhizopus stolonifer*, *Aspergillus fumigatus*, *Aspergillus niger*, *Fusarium oxysporum*, and *Trichoderma* sp., isolated from sanitary pads (Lawal, O.B., 2023). Another study on the microbial quality of sealed tampons reported the presence of *Bacillus subtilis*, *Staphylococcus epidermidis*, *Bacillus licheniformis*, *Bacillus pumilus*, *Micrococcus luteus*, and *Alicyclobacillus acidoterrestris*, along with a few fungal isolates (Briancesco, R., 2018).

The isolated bacteria comprised *S. aureus*, *Bacillus* spp., *E. coli*, *Enterobacter* spp., *Klebsiella spp.*, and *Pseudomonas aeruginosa*. Among these, *Bacillus cereus* and *Staphylococcus aureus* are part of the normal oral and skin flora (Marsh et al., 2016; Zhou et al., 2021), yet they have also been identified as pathogens in certain cases of bacterial vaginosis (Salliss et al., 2021). *Staphylococcus aureus* is associated with various vaginal infections and Toxic Shock Syndrome (TSS) (Stewart & Spencer, 2022). Coliforms such as *E. coli*, which are part of the normal intestinal flora, have the capacity to asymptomatically colonize the vagina and may act as opportunistic and potentially deadly pathogens. Common clinical conditions caused by *E. coli* in females include urinary tract infections (UTIs) and pelvic inflammatory disease (PID). If untreated, these infections may lead to severe pregnancy complications (Cools, 2017).

Unlike *E. coli*, *Enterobacter* spp. and *Klebsiella pneumonia* are also known uropathogens and can cause UTIs (Ramirez & Girón, 2023; Marques et al., 2019). On the other hand, *P. aeruginosa*, a well-known opportunistic pathogen, has been co-isolated with *E. coli* and *S. aureus* in cases of bacterial infections of the female genital tract and has also been linked to PID (Vander & Prabha, 2019). The establishment of infection depends on multiple factors, including the microbial load, the ability of pathogens to modulate host immune responses, and environmental conditions such as vaginal pH and humidity (Galli et al., 2004; Bergstrom et al., 2012).

3.2 Identification of the Antibiotic Resistant bacterial isolates.

The present study also aimed to identify antibiotic-resistant bacterial isolates. Therefore, all probable bacterial isolates were subjected to Antibiotic Susceptibility Testing (AST) using broad-spectrum antibiotics. Out of the 34 probable bacterial isolates, 8 were found to be antibiotic-resistant, as shown in Table 3. Among these, 3 were strains of *S. aureus*, and 2 were *E. coli* strains. A strain of *S. aureus* resistant to both penicillin G (10 µg) and streptomycin (10 µg) was isolated from Sofy sanitary pads, while the remaining resistant strains were isolated from sanitary pads available in loose packing without a brand name.

Table 3: Antibiotic Resistant bacterial isolates form the sanitary pads

Strain No.	Name of the isolate	Resistant to the antibiotic
1	*S. aureu*	Tetracycline (TE-30)
2	*S. aureus*	Penicillin G (10µg)
3	*S. aureus*	Penicillin G (10µg) and Streptomycin (10µg)
4	*B. cereus*	Ampicillin (10 µg)
5	*E.coli*	Tetracycline (TE-30)
6	*E.coli*	Tetracycline (TE-30) and Chloramphenicol (30µg)
7	*Enterobater spp.*	Tetracycline (TE-30) and Penicillin G (10µg)
8	*Klebsiella spp.*	Ampicillin (AMP-10)

The 2022 report by the Global Antimicrobial Resistance and Use Surveillance System (GLASS) highlights alarming resistance rates among prevalent bacterial pathogens. According to this report, there is an increasing prevalence of cephalosporin-resistant *E. coli* and methicillin-resistant *Staphylococcus aureus* (MRSA) in 76 countries, which remains a major public health concern. The report also states that one out of five cases of urinary tract infections (UTIs) caused by *E. coli* in 2020 exhibited reduced susceptibility to standard antibiotics such as ampicillin, co-trimoxazole, and fluoroquinolones, thereby complicating effective treatment (GLASS Report, 2022).

4 CONCLUSION

The present analysis demonstrates that sanitary pads can harbor bacterial contaminants due to the ubiquitous presence of microorganisms in the environment, particularly when manufacturing, storage, or handling conditions are unhygienic. The detection of potentially pathogenic bacteria raises significant public health concerns, especially for women in low-resource settings who may rely on unregulated products. These findings underscore the urgent need for stringent quality control measures, routine microbiological testing, and robust regulatory oversight in the production and packaging of sanitary pads. Each country should establish a strong surveillance system to monitor the production and quality of sanitary products available on the market. Furthermore, promoting awareness of proper usage,

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avoiding prolonged wear, and maintaining personal hygiene is essential. With adequate education and hygienic practices, sanitary pads can be used safely without compromising women's reproductive health.

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