

A study on the microbial quality of sealed products for feminine hygiene

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Keywords

Microbial flora • Safety • Tampons

Summary

Introduction. Sanitary tampons have been in existence for over 60 years. Their use may present certain health risks, potentially associated with an abnormal change of microbial flora in the vagina (e.g., toxic shock syndrome). Tampon production and marketing are regulated differently in different countries. In Australia, Canada and the USA, tampons are classified as Class-II medical devices and their marketing requires pre-clinical and clinical studies, including microbiological trials. In Europe, tampons are considered consumer products and safety-related data are provided only if the manufacturer deems them to be useful. Sterility of these products is not requested by law; thus they may represent a potential vehicle for microorganisms. Due to the lack of data on microbial characteristics of tampons, an analytical investigation was carried out to characterize and quantify the microbial

flora present on sealed tampons of various brands present on the market in Italy.

Methods. Traditional cultural methods were used to characterize and quantify bacteria and fungi. Identification of colonies was performed with biochemical techniques.

Results. Results showed low microbial concentrations in 93% of the positive samples. A rare presence of opportunistic pathogens was detected and a few samples (6%) were characterized by bacterial species of human origin.

Conclusions. In the light of these data, the examined tampons were found to have good hygienic quality. Nevertheless, to minimize the microbial risks linked to the use of these products, strict hygienic rules during their production and manipulation have to be adopted.

Introduction

The introduction on the international market of sanitary towels, also known as internal tampons, dates back to the thirties of the last century. The spread of tampon use, initially slowed by misinformation and some initial injuries, had a dramatic increase once its advantages over conventional sanitary tampons became apparent. The reassurance of freedom of movement in performing all types of work and sports during menstruation, combined with their protection and comfort are the characteristics that for over sixty years encouraged many women to use these products.

The function of the internal tampon is to absorb menstrual blood inside the vagina after it has left the uterus, preventing it from leaking out, and thus providing suitable protection with total discretion. The differences in the use of external tampons requires some knowledge of female genital tract anatomy for the tampon to be properly positioned, a prerequisite in ensuring total absorption. The choice available between the different tampon types with different absorption characteristics depends on the required level of protection, according to different needs.

Currently, internal tampons usually consist of an absorbent cellulose material and plastic derived material. They can be wrapped by a thin non-woven fabric layer

and, at times, with an applicator in plastic or cardboard, and a cotton, polyethylene or viscose string for extraction [1]. These products are not requested to be sterile.

Over the years there were some problems related to the use of tampons, some, less serious, being vaginal dryness and ulcerations of the vagina, usually associated with the use of tampons with absorbency higher than the required needs [2]. Far more important however, the Toxic Shock Syndrome (TSS) can appear as a severe toxemia, sometimes rapidly becoming fatal. Its symptoms are high fever, vomiting, diarrhea, confusion and rash [3]. The disease is caused by the *Staphylococcus aureus* toxin 1 (TSST-1), bacterium that commonly colonizes nose and vagina [4]. The women most at risk of TSS are those with an earlier colonization of the vagina, regularly using tampons. It is likely that mechanical or chemical factors related to the use of tampons favor an increase of the bacterial toxin production which enters the bloodstream through the disruption of the mucosa [5]. The incidence of this disease in women has rapidly decreased following massive advertising campaigns on the role of the tampons and diaphragms, and after the withdrawal of some brands of tampons from the market [6]. According to recent estimates, the incidence of the disease is 3 cases per 10,000 menstruating women [7]. Moreover there is evidence that in some cases this also occurs in women not using tampons, and also in the post-operative

period and post-partum situations [8]. During use, the risk was related mainly to prolonged times of use (more than eight hours) [9]. Based on the results of some researches, an increase of glucose concentration, due to lysis of the carboxymethyl cellulose by the microbial flora of the vagina, can support the growth of *S. aureus* [10]. Regulations on production and marketing of tampons differ across countries, and are stricter in Canada, Australia and the United States [11, 12]. Internationally, there is no legislation providing for sterility requirements.

In the United States these products are classified as Class II medical devices. Special controls are required, and their put on the market is subject to the approval of the Food and Drug Administration [12]. In addition to requirements for shape, size, composition, absorption capacity and presence of chemical residues, the manufacturer must perform pre-clinical toxicological and microbiological tests. Particularly, for microbiological safety requirements, the manufacturer must demonstrate that the product does not favor the growth of *S. aureus*, does not stimulate the production of TSST-1 toxin, and does not alter the normal vaginal microbial flora. Clinical studies are also required if the products have new types of buffers, or when there are significant changes in the design and material used, compared to the traditional sold tampons [12].

In the European Union, tampons are not considered medical devices and therefore are not regulated as such. The producer can decide whether clinical trials are required to verify the safety of the product [13, 14]. In Italy, tampons, as other products for feminine hygiene, are consumer goods, and the safety obligations of the manufacturer and distributor are defined by the Consumer Code [15].

Given the mode of use, as well as providing a possible growth support for the microorganisms present on vaginal mucosa, tampons can be a vehicle of exogenous microorganisms in the female genital apparatus.

Numerous clinical studies have been performed to characterize the microbial vaginal flora during menstruation and to evaluate any change of its composition associated with the use of tampons [16, 17]. Many studies have also focused on the possible role of the chemical components of tampons as a potential support for microbial growth [10, 18]. Instead, currently, no data exist to estimate the possible impact in terms of the microbial concentration and type of species conveyed by non-sterile exogenous devices on the vagina.

Therefore an analytical investigation was performed to quantify and characterize the microbial flora present on tampons of various brands present on the Italian market.

Methods

Fifteen brands of sealed internal tampons from five major manufacturers were subjected to microbiological analysis. The products were aseptically removed from their packaging, immersed in 100 mL of buffered saline

solution added and stirred on a rotary plate for 2 minutes. The concentrated eluate was divided into several aliquots and the analyses were performed using the membrane filtration technique. Eight different groups of microorganisms/bacterial species were investigated. Thus, the membranes were incubated on various agarized media for the detection of the following microbial parameters:

- *Mesophilic bacteria*. Incubation on Plate Count Agar (Oxoid/ThermoFisher, USA) at 36°C for 72 h; the count of all colonies was made.
- *Fungi (molds) and yeasts*. Incubation on Sabouraud Destrose Agar (ThermoFisher Diagnostics, USA) at 25°C for 7-10 days; mold and yeast colonies were counted.
- *Anaerobic Bacteria*. Incubation on Plate Count Agar (ThermoFisher Diagnostics, USA) at 36°C in anaerobiosis for 72 h; the count of all colonies was made.
- *Coliforms*. Incubation on C-EC (Biolife, Italy) at 36°C for 24 h; blue colonies were counted.
- *Escherichia coli*. Incubation on C-EC (Biolife, Italy) at 36°C for 24 h; blue and fluorescent colonies were counted using a Wood lamp.
- *Staphylococcus spp.* Incubation on Baird Parker (ThermoFisher Diagnostics, USA) at 36°C for 48 h; the count of black colonies was made.
- *Candida albicans*. Incubation on Biggy Agar (ThermoFisher Diagnostics, USA) at 36°C for 18-72 h; dark brown colonies were counted.
- *Pseudomonas aeruginosa*. Incubation on Pseudomonas Agar/CN (ThermoFisher Diagnostics, USA) at 36°C for 48 h; the count of green-blue colonies was made, and a biochemical confirmation of fluorescent and reddish brown colonies using a Wood lamp was performed.

Biochemical identification: the grown bacterial colonies were isolated and identified by the miniaturized system VITEK® 2 Compact (Biomerieux, France).

Results

From the 15 tampons examined, only one did not show microbial growth, while 93% of the samples exhibited a moderate bacterial load (Tab. I). A maximum of 100 cfu/tampon for the mesophilic bacteria and 55 cfu/tampon for anaerobic bacteria were counted, respectively. Molds were detected in 27% of the tampons, and the highest mold load did not exceed 15 cfu/tampon. Yeasts were absent in all samples. None of the examined tampons showed contamination by the fecal bacterial indicators, *E. coli* and Coliforms. The species *P. aeruginosa* and *S. aureus* were also absent in all samples, as well as *Candida albicans*.

From all the positive samples for the mesophilic bacteria, species belonging to the genus *Bacillus* were isolated. Bacteria of the genus *Alicyclobacillus* were also identified in one sample, and in 50% of the samples, *Staphylococcus epidermidis* and *Micrococcus luteus* were detected (Tab. II).

Tab. I. Results of the microbiological analysis of the tampons.

Product code	Mesophilic bacteria	Molds	Anaerobic bacteria	<i>Staphylococcus</i> spp.
	CFU/tampon	CFU/tampon	CFU/tampon	CFU/tampon
1T	10	< 1	< 1	5
2T	20	5	< 1	< 1
3F	100	10	< 1	< 1
4N	100	5	< 1	< 1
5O	40	15	15	< 1
6C	5	< 1	55	< 1
7C	20	< 1	< 1	< 1
8O	10	< 1	< 1	< 1
9T	10	5	< 1	< 1
10V	5	< 1	< 1	< 1
11O	100	< 1	5	< 1
12T	10	< 1	< 1	< 1
13L	100	< 1	< 1	< 1
14T	5	10	< 1	< 1
15T	< 1	< 1	< 1	< 1

CFU: Colony Forming Unit

Tab. II. Bacterial species identified from the analysed tampons.

Product code	Identified species
1T	<i>Bacillus subtilis</i> <i>Staphylococcus epidermidis</i>
2T	<i>Bacillus subtilis</i> <i>Bacillus licheniformis</i>
3F	<i>Bacillus pumilus</i>
4N	<i>Bacillus licheniformis</i> <i>Bacillus subtilis</i>
5O	<i>Alicyclobacillus acidoterrestris</i> <i>Bacillus licheniformis</i> <i>Bacillus subtilis</i>
6C	<i>Bacillus subtilis</i>
7C	<i>Bacillus circulans</i>
8O	<i>Bacillus subtilis</i>
9T	<i>Micrococcus luteus</i> <i>Bacillus subtilis</i>
11O	<i>Bacillus pumilus</i>
12T	<i>Micrococcus luteus</i>
13L	<i>Bacillus pumilus</i> <i>Bacillus licheniformis</i>
14T	<i>Bacillus circulans</i>

Discussion and conclusions

As well as other parts of the human body, the vagina is populated by numerous microorganisms which together constitute the vaginal microbiome. This ecosystem is typical model of well-organized balanced mutualistic consortium. The indigenous bacterial communities play a protective role in preventing colonization of host by opportunistic pathogens.

Lactobacillus is the dominant vaginal bacterial genus and, to a lesser extent, streptococci, enterobacteria, staphylococci, corynebacteria, anaerobic bacteria, *Gard-*

nerella, *Candida* and *Mycoplasma* even colonize this district. Some of these, whilst being potential pathogens, do not represent a real health risk, unless their concentrations increase in a non-proportional manner, attributable to imbalances due to various causes. Given the lack of sterility, potential inadequate measure of hygiene during the insertion, anatomical proximity of the genito-urinary system and intestinal apparatus, the use of internal tampons could be a potential vehicle for microorganisms.

However, the data obtained from the quantification and characterization of the microbial populations of these products showed not high concentrations of bacteria and molds.

As for the composition of the microbial flora present onto these products, the most common microorganisms were bacteria belonging to the genus *Bacillus*, known to be present ubiquitously in nature, to produce endospores, to be capable of withstanding particularly hostile environmental conditions. The isolated species from tampons, namely *B. subtilis*, *B. circulans*, *B. licheniformis* and *B. pumilus*, are not generally associated with pathological conditions, although the latter two have been reported as agents responsible for diseases in immunocompromised individuals [19].

Staphylococcus spp. and *Micrococcus* spp. were also isolated. Species belonging to these genera are common in human environments and on human body. In particular, *Staphylococcus epidermidis* is a member of the normal human cutaneous and mucosal flora and represents the 65-90% of all staphylococci that usually inhabit skin, vagina, urethra and mouth. In physiological conditions, the bacterium does not harm the host. However, in an impaired immune condition (undergoing surgical implants or transplants etc.), this species can become a commensal opportunistic pathogen, causing disease in immunocompromised and catheterized individuals [20]. Even *Micrococcus luteus* is part of the skin microbiome of mammals and is a ubiquitous species in the environment. It is not considered a pathogen, but an opportunist bacterium, responsible for skin infections, endocarditis, septicemia and septic shock [21].

Taking into account both the scattered detection of the bacterial species of human origin (6% of the samples) and their low microbial loads, the presence of these bacteria in the examined sealed internal tampons may not constitute a health risk to the consumer. Only in specific host immune debilitation conditions and at high concentrations these microorganisms can perform their pathogenic action. This aspect was taken into consideration when 1400 tampons were recalled because the company tests detected *Chronobacter sakazakii* on the plastic tubing [22]. In fact, this bacterium, responsible of vaginal and urinary tract infections, pelvic inflammatory diseases or other potentially life-threatening infections, represents a higher risk in immunosuppressed women.

In view of our results, for minimizing the risks of the microbial origin, next to strict hygienic rules during production and packaging of these products, the observance of good practices (e.g., hand washing) during their use

and manipulation has to be adopted for reducing potential allochthonous contamination.

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Conflict of interest statement

None declared.

Authors' contributions

All authors discussed the data and results and contributed to the final manuscript.

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