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Spoilage Bacteria Associated with Selected Body Lotions Commonly Used amongst Students of the University of Port Harcourt, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author EE designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors HOS and CNS managed the analyses of the study. Author CNS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The study investigated the spoilage bacteria associated with selected body lotions commonly used amongst students of the University of Port Harcourt. The influence of the body lotion on the bacterial composition of the skin was also determined. Five in- use lotions were obtained from students within the University while five unused ones were purchased from stores. Skin swabs were collected before and after use of the lotion from 25 different study subjects for a period of 10 consecutive days. Bacterial counts were determined using the spread plate method. Average bacterial counts of skin swab ranged from 6.0±0.5 to 300.0±0.0 and from 2.0±0.0 to 300.0±0.0. Total heterotrophic bacterial counts of in-use and unused lotion samples ranged from 5.84 log cfu /ml to 7.14 log cfu/ml and from 5.50 log cfu/ml to 7.04 log cfu/ml respectively. Total Staphylococcal

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counts ranged from 4.76 log cfu/ml to 6.39 log cfu/ml and from 4.27 log cfu/ml to 5.57 log cfu/ml respectively while total coliform counts ranged from 0 log cfu/ml to 5.88 log cfu/ml and from 0 log cfu/ml to 3.47 log cfu/ml respectively. Total *Shigella* counts obtained from used lotion samples ranged from 0 log cfu/ml to 5.54 log cfu/ml while it was not detected in the unused samples. The bacterial organisms isolated from the study include; *Escherichia* spp*, Bacillus* spp*, Staphylococcus* spp, *Proteus* sp *and Micrococcus* sp. The study also showed incomplete label disclosure. The study revealed the presence of bacteria of public health importance in both the in-use and unused lotion samples. The need for stricter adoption and maintenance of current good manufacturing practices during manufacture and better hygiene practices during usage by consumers cannot be overemphasized.

Keywords: Spoilage bacteria; body lotion; coliform; Staphylococcus aureus; Escherichia coli.

1. INTRODUCTION

Cosmetics are either chemical or natural preparations which humans apply to body parts specifically for beautifying, cleansing and protecting the skin [1]. Many of the cosmetic products currently available in the market contain additives such as plant extracts, fatty acids and vitamins. These additives incorporated into cosmetic products supposedly to nourish the skin may also serve as nutrients that support the growth of microorganisms and thus pose a risk to the consumer. Microorganisms have over the years demonstrated the ability to grow and reproduce in cosmetics and allied products resulting in spoilage or chemical /physical deterioration to the cosmetic product while causing harm to the user [2-5]. Although cosmetics do not belong to the category of sterile products, they are however, expected to be completely devoid of virulent pathogenic microbes with very low aerobic microbial load. With respect to numbers, only temporary guidelines are in force since there are yet no generally acceptable standards. Products for use around the eye should not contain more than 500 colony forming units (CFU)/g while for non-eye-area products, counts of not more than 1000 CFU/g may be allowed [6]. Several spoilage bacteria have been noted to cause product contamination. Such pathogens or opportunistic pathogens whose occurrence particularly in cosmetic products for use in the eye area, may elicit concern include *Staphylococcus aureus, Pseudomonas aeruginosa*, *Streptococcus pyogenes, Klebsiella pneumoniae* and some Gram negative bacteria [6,7]. Cosmetics though not sterile, contain antimicrobial preservatives that are supposed to help them withstand a certain degree of in–use abuse by consumers. In spite of this, microorganisms still get introduced into cosmetic products as contaminants, probably accidentally, either during manufacture or during use by consumers. These microbial contaminants of cosmetic products can be isolated using the method of direct colony counts or enrichment culturing inactivating the preservative system often by dilution or by use of specific neutralizers. The isolated microbes can then be identified by means of routine microbiological methods or by the use of commercial identification kits [6].

This study therefore set out to determine the incidence of microbial contamination in some commonly used cosmetics lotions amongst students of the University of Port Harcourt, Nigeria.

2. MATERIALS AND METHODS

2.1 Collection of Samples

In-use body lotion samples were obtained from students in the University of Port Harcourt and unused lotion samples were bought commercially within same area. Samples were obtained from the study participants and immediately transported to the microbiology laboratory for immediate analysis.

2.2 Media Used

Nutrient agar, MacConkey agar, Mannitol salt agar, Salmonella and Shigella agar and Peptone water were used in the isolation and determination of the bacterial load of the sample. The media were reconstituted and sterilized according to the manufacturer's instruction.

2.3 Skin Swab Analysis

Sterile swab sticks were swabbed on posterior parts of the forearms of twenty five students who had used the five lotion samples being studied.

The exercise was carried out for a period of 10 consecutive days and swabs were collected each day after bathing before using the lotion, and 30 minutes after using lotion. Participants were advised not to use towels but expose themselves to atmospheric air. A total of 500 swabs were obtained in the study and characterized for total heterotrophic bacteria count. The swab sticks were suspended in 10 ml peptone water contained in Bijou bottle and allowed to stand for 30 minutes after vigorous shaking. A 0.1 ml volume was then withdrawn and plated out on the surface of sterile plates containing nutrient agar using the spread plate method for the enumeration of bacterial count.

2.4 Bacterial Counts of the Body Lotion

A 1 ml volume of each lotion sample was introduced into 4 ml sterile Ringer solution which contained 0.25% tween 80. Ten-fold serial dilutions of the samples were made in the same diluent up to 10^4 and 0.5 ml was plated out on the solid media mentioned above. The plates were incubated for 24 hours at 37°C and the resultant colonies were counted. All operations were carried out in triplicates and the results were expressed as colony forming unit per millilitre (CFU/ml).

2.5 Identification of Bacterial Isolates

All bacterial isolates were identified based on their Gram reaction and biochemical tests [6,8].

2.6 Statistical Analysis

All results were analysed statistically using SPSS mini tab (version 2.0).

3. RESULTS

Table 1 shows label disclosure details of lotion samples; Table 2 shows percentage (%) response of study subjects to used lotions while Table 3 shows total viable counts of bacterial isolates from the skin swabs of University respondents using a brand of cosmetic body lotion.

Fig. 1 shows total viable bacterial count of in-use and unused lotion samples collected from participating students. Counts were higher in used samples compared to unused ones as seen in Fig. 1.

Table 4 shows biochemical characteristics of bacterial isolates and Table 5 shows types of bacteria isolated from in-use and unused lotions.

4. DISCUSSION

The high bacteria counts recorded after use of lotion samples implies that lotion may have served as nutrient for bacterial growth. Vitamins and plant extracts incorporated in many lotions are nutrients and could enhance growth of microorganisms already present on the skin. The incorporation of additives may have promoted reduction in bacterial population for the only one reported before use of lotion sample.

Perspiration would possibly support microbial growth while microorganisms such as *Bacillus* and *Staphylococcus aureus* have been linked with skin irritation [8]. The high level of perspiration and skin irritation recorded in this study is in agreement with this finding.

Present study showed that the manufacturers of lotion samples examined did not comply with the standards stipulated by International Microbiological Standards (IMS). The IMS recommended that bacterial load in cosmetics should not exceed 1.0 x 10^3 cfu/ml and coliform must totally not be present [9]. Only three samples complied with the recommended coliform standard.

Table 1. Label disclosure details of lotion samples

Samples	Manufacturing date	Expiry date	NAFDAC NO.	Batch no.	Manufacturer's address
A	+	+	+	+	+
B	-	-	+	+	-
C	+	+	-	+	-
D	+	+	+	+	+
E	+	+	+	-	+

Key + = Present, - =Absent

Table 2. Percentage (%) response of study subjects for used lotions

Reaction	Yes	No
Profuse perspiration	72	28
Rash	12	88
Itch	60	40
Change in Colour	4	96

The high bacteria counts noticed in unused lotion samples suggests the absence of or non-adherence to current good manufacturing practices (cGMP) which may include use of contaminated water during production and staff negligence during packaging. The elevation in bacteria number for in-use lotion samples may be linked to unhygienic practices by consumers and improper storage after use. A survey done during the study showed that some consumers tend to transfer excess lotion back into the containers from their hands which could lead to the introduction of contaminants. Others tend to leave the containers open for a long time after use allowing air into it and this could support the proliferation of aerobic microorganisms.

The results of present study were in agreement with that of [10], who reported *Staphylococcus* sp as most predominant in their study on lotions and creams. *Staphylococcus aureus* is a normal flora of the skin and its appearance in high number in lotion samples might have resulted from its shedding from skins of handlers and manufacturers of these lotions. This result differs from that of [11] who did not isolate *Escherichia coli* in their study. The dominance of *Escherichia coli* in in-use samples points to pollution and possibly the presence of pathogenic microorganisms. It also suggests a high level of carelessness on the part of consumers.

Table 3. Total viable counts of Bacterial Isolate from the skin Swabs of University respondents using a brand of Cosmetic body lotion.

Day	Period of use	A	B	C	D	E
1	Before	6.0 ± 0.5 ^{b}	16.6 ± 0.8 ^{a}	14.0 ± 1.1 ^{b}	300.0 ± 0.0 ^{a}	192.0 ± 0.5 ^{a}
	After	2.0 ± 0.0 ^{a}	21.0 ± 0.5 ^{b}	3.0 ± 0.5 ^{a}	300.0 ± 0.0 ^{a}	204.0 ± 1.1 ^{b}
2	Before	24.0 ± 0.5 ^{b}	28.0 ± 0.5 ^{a}	12.0 ± 0.5 ^{a}	19.0 ± 0.5 ^{a}	42.0 ± 0.5 ^{b}
	After	20.0 ± 0.5 ^{a}	61.0 ± 0.5 ^{b}	11.0 ± 0.5 ^{a}	33.0 ± 0.5 ^{b}	31.0 ± 0.5 ^{a}
3	Before	172.0 ± 0.5 ^{a}	76.0 ± 1.1 ^{a}	168.0 ± 1.1 ^{a}	216.0 ± 1.1 ^{a}	109.0 ± 1.1 ^{b}
	After	169.0 ± 1.1 ^{a}	189.0 ± 1.1 ^{b}	248.0 ± 1.1 ^{b}	300.0 ± 0.0 ^{b}	61.0 ± 1.1 ^{a}
4	Before	279.0 ± 1.1 ^{b}	91.0 ± 1.1 ^{a}	222.0 ± 0.5 ^{a}	118.0 ± 1.1 ^{a}	51.0 ± 1.1 ^{a}
	After	188.0 ± 1.1 ^{a}	211.0 ± 1.1 ^{b}	256.0 ± 1.1 ^{b}	286.0 ± 1.1 ^{b}	86.0 ± 1.1 ^{b}
5	Before	294.0 ± 1.1 ^{b}	79.0 ± 1.1 ^{a}	312.0 ± 1.1 ^{b}	74.0 ± 1.1 ^{a}	84.0 ± 1.1 ^{a}
	After	197.0 ± 1.1 ^{a}	263.0 ± 1.1 ^{b}	291.0 ± 1.1 ^{a}	199.0 ± 1.1 ^{b}	113.0 ± 1.1 ^{b}
6	Before	173.0± 1.1 ^{b}	72.0±1.1 ^{a}	242.0±1.1 ^{a}	61.0±1.1 ^{a}	79.0±1.1 ^{a}
	After	91.0±1.1 ^{a}	223.0±1.1 ^{b}	281.0±1.1 ^{b}	271.0±1.1 ^{b}	134.0±1.1 ^{b}
7	Before	194.0 ±1.1 ^{b}	87.0±1.1 ^{a}	253.0±1.1 ^{a}	42.0±1.1 ^{a}	113.0±1.1 ^{a}
	After	112.0±1.1 ^{a}	276.0±1.1 ^{b}	266.0±1.1 ^{b}	211.0±1.1 ^{b}	242.0±1.1 ^{b}
8	Before	212.0±1.1 ^{b}	94.0±1.1 ^{a}	239.0±1.1 ^{b}	77.0±1.1 ^{a}	49.0±1.1 ^{a}
	After	133.0±1.1 ^{a}	286.0±1.1 ^{b}	211.0±1.1 ^{a}	242.0±1.1 ^{b}	132.0±1.1 ^{b}
9	Before	263.0±1.1 ^{b}	89.0±1.1 ^{a}	311.0±1.1 ^{b}	63.0±1.1 ^{a}	52.0±1.1 ^{a}
	After	152.0±1.1 ^{a}	266.0±1.1 ^{b}	289.0±1.1 ^{a}	187.0±1.1 ^{b}	122.0±1.1 ^{b}
10	Before	271.0±1.1 ^{b}	79.0±1.1 ^{a}	286.0±1.1 ^{b}	67.0±1.1 ^{a}	63.0±1.1 ^{a}
	After	163.0±1.1 ^{a}	281.0±1.1 ^{b}	211.0±1.1 ^{a}	246.0±1.1 ^{b}	131.0±1.1 ^{b}

Legends: Before – skin swabs taken after bathing/before use of lotion
After – skin swabs taken after bathing/after use of lotion
Means with same superscripts (a or b) are statistically insignificant (P > 0.05)
Means with different superscripts (a and b) are statistically significant (P < 0.05)
A, B, C, D, E – Lotion samples used by the subjects

Table 4. Biochemical Characteristics of Bacterial Isolates

Gram reaction	Shape	Spore	Motility	Catalase	Oxidase	Citrate	Indole	MR	VP	H_2S	Slant	Butt	Gas	Glucose	Sucrose	Lactose	Probable organism
+	Cocci	-	-	+	-	+	-	+	+	-	B	A	+	-	+	-	*Micrococcus* sp
-	Rod	-	+	+	-	-	+	+	-	-	B	A	+	+	-	-	*Proteus* sp
+	Rod	+	-	+	-	+	-	-	-	-	B	A	-	+	+	-	*Bacillus* sp
+	Rod	+	+	+	-	+	-	-	+	-	B	A	-	+	-	-	*Bacillus* sp
-	Rod	-	+	+	-	-	+	+	-	-	A	A	+	+	+	+	*Escherichia* sp
+	Cocci	-	+	+	-	+	-	-	+	-	A	B	-	+	-	+	*Staphylococcus*sp

+ = Positive,- = Negative, A = Acidic and B = Alkali

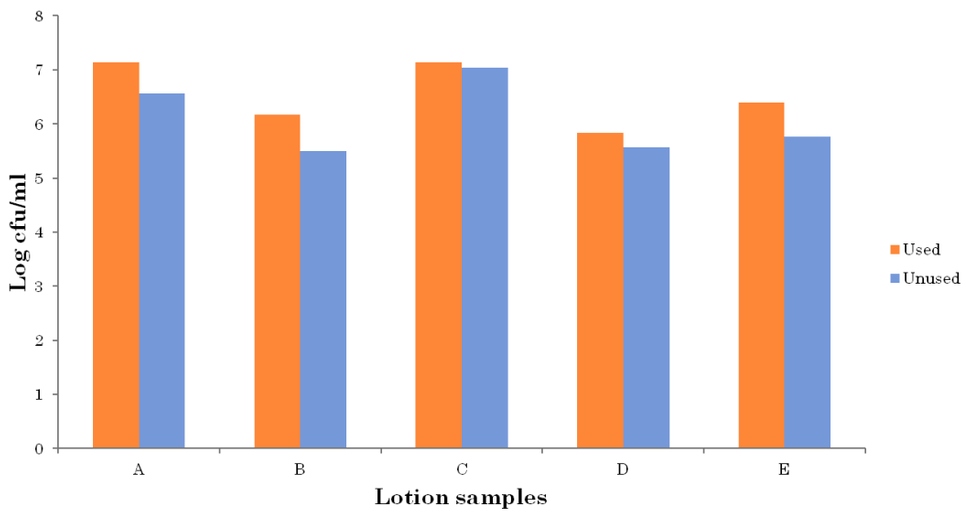

Fig. 1. Total viable Bacterial count of the in-use and unused lotion samples

The present study analysis of label information is in agreement with that of Mwambete and Simon [12] who also established a lack of complete label information on products to guide consumers.

Table 5. Types of bacteria isolated in used and unused lotions

S/n	Organism	Used	Unused
1	*Escherichia* sp	+	+
2	*Bacillus* sp	+	–
3	*Bacillus* sp	+	+
4	*Micrococcus* sp	–	+
5	*Staphylococcus* sp	+	+
6	*Proteus* sp	+	–

+ = Present, –Absent

5. CONCLUSION

Results of the study here have clearly shown that lotion samples were contaminated by spoilage bacteria irrespective of date of expiration (for the ones provided). The study also revealed that unused lotion samples did not meet stipulated bacteriological standards implying that the first point of contamination was in the production process. Compared to the unused samples, the bacterial load of in-use lotion samples were generally higher. It does appear that unhygienic practices by users may also have contributed to the presence of these organisms in the lotion samples. Even though cosmetics are not sterile products, they are however, required to conform to acceptable minimum standards of microbial load. The prevalence and types of pathogens seen in the cosmetics tested as revealed by this study is clearly unacceptable and pose a great risk to public health. Therefore, the National Agency for Food and Drug Administration (NAFDAC) and other statutory regulatory bodies should intensify control and monitoring efforts to ensure that the consuming public is protected from the deleterious effects of fake, sub-standard and unwholesome products.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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