

MICROBIOLOGICAL PROFILE OF COMMONLY AVAILABLE COSMETICS AND TOILETRIES IN NSUKKA, NIGERIA

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ABSTRACT

Microbiological analysis was carried out on a total of forty eight (48) cosmetics and toiletry preparations made up of three classes of products – Relaxers, Body creams and Toothpastes. Six brands each of relaxers and creams and four brands of toothpastes were analysed in a manual plate count method using three samples of each brand. None of the relaxer samples contained viable bacteria but fungi were isolated from four of the six samples. Two cream samples and two toothpaste samples yielded viable counts of bacteria ranging from 221 cfu g⁻¹ and 120 cfu g⁻¹ for the creams to 117 cfu g⁻¹ and 96 cfu g⁻¹ for the toothpastes. Bacteria isolated include members of the genera *Bacillus*, *Pseudomonas*, *Staphylococcus* and *Lactobacillus*. Fungi isolated were species of *Mucor*, *Geotrichum*, *Curvularia*, *Trichophyton*, *Aspergillus*, *Fusarium* and *Cladosporium*. Antibiotic susceptibility assay revealed that 8% of the *Staph aureus* isolates were resistant to erythromycin, streptomycin and ampiclox while 33% were resistant to lincomycin. *Pseudomonas* sp presented the highest resistance of 55% to ampicillin. The pH of the samples showed that the relaxers were alkaline with a pH range of 10.2 to 12.2, the toothpastes were slightly acidic and the creams slightly acidic to near neutral.

KEYWORDS: Cosmetics, Toiletries, bacteria, fungi, antibiotic resistance

INTRODUCTION

Majority of cosmetic products are susceptible to microbial contamination (Cremieux *et al* 2005). In some instances, microorganisms in cosmetics and toiletry preparations, whether present initially or transferred to the product by the users have been implicated as the aetiological agents of diseases (Jarvis *et al* 1974). Changes and deterioration in cosmetics products can be brought about by changes in acidity or alkalinity or as a result of hydrolysis, oxidation, heat, light, bacterial or fungal contamination and metabolic activities (Smart and Spooner, 1972). Many products contain preservatives which kill or inhibit the growth of microorganisms but upon storage, the effectiveness of such preservatives can be reduced by chemical changes. On application to the body, the effectiveness can be reduced due to dilution with moisture or even by evaporation of the preservatives. Previously inhibited organisms can then grow and become potentially pathogenic if they gain entry into the body (Evans *et al* 1972). In the past few years increasing interest has been shown in the susceptibility of cosmetics and toiletry products to microbial contamination and the potential risk of infection to the users of those products (Baird, 1977). A number of workers have attempted to determine the incidence of contamination and by extension the efficacy of antimicrobial preservatives (Guitton *et al* 2005; Cremieux *et al* 2005). The results from these surveys are conflicting and contamination rates have been found to vary from 2.5% to 43% (Wilson *et al* ,1971; Myers and Pasutto, 1973, Jarvis *et al* ,1974; CTFA, 1975). There is even less information on the possible health risk to the user of contaminated products. The available information, as scanty as it is, relates mostly to what is obtainable in developed countries of the world. There is a paucity of literature describing microbial bioburden of cosmetics and toiletries in Nigerian markets. This paper is therefore aimed at presenting the results of an investigation into the microbial contamination of cosmetic and toiletry products in Nsukka, Nigeria.

MATERIALS AND METHODS

Three samples each of sixteen different (brands) products from different retail outlets in Nsukka were purchased as packaged by the manufacturers and subjected to microbiological analysis. Products were inspected to detect leaks and irregularities in shapes of containers.

Media and Diluent: Media used are nutrient agar (Oxoid) MacConkey agar, Sabouraud Dextrose Agar (Oxoid) and Mueller Hinton agar (Oxoid). One diluent – Tween – peptone was used for all the analytes and the composition was 0.1% w/v peptone solution (pH 7.0) containing 0.1% v/v Tween 80, prepared according to the methods of Jarvis *et al* (1974).

Determination of pH of Samples: Test solutions (25mg/ml) of samples were assayed for their pH reading using an electric pH Meter Model 290mk of Pye Unicam.

Sample preparation and Microbiological Procedures: The outside surfaces of all containers were swabbed with 70% v/v ethanol before opening. One gram of each product was homogenized in 20ml sterile diluent using a vortex mixer for 10 minutes. An aliquot (0.1ml) of each of these preparations was spread individually and uniformly over the surface of previously prepared and dried plates of nutrient agar, MacConkey agar and Sabouraud Dextrose agar. Surface plating method, rather than pour plating was used to avoid the possibility of killing stressed organisms with molten agar in accordance with the method of Palmieri (1983). The nutrient agar and MacConkey agar plates were incubated at 30°C and 37°C respectively for 24hr while the SDA plates were incubated at room temperature for 5 – 7 days. Counts of bacteria and fungi were made on nutrient agar and SDA respectively after appropriate incubation periods. Representative colonies were subcultured and subsequently subjected to identification procedures. Fungal contaminants were identified by colonial and cellular morphology according to the methods of Evans and Richardson (1989), and Beneke (1974).

Colonies of bacteria were Gram-stained and further analysed for their biochemical and physiological characteristics using the methods of Cowan and Steel (1984) and identified based on the criteria of Krieg and Holt, (1984).

Antimicrobial susceptibility tests: Using the methods of Bauer *et al* (1966), the bacterial isolates were assayed for their sensitivities to the following Optun Laboratory antibiotic paper disks: gentamycin (10µg), lincocin (30µg), rifampin (10µg), erythromycin 30(µg), chloramphenicol (20µg), streptomycin 30(µg), ampiclox 30(µg), co-trimoxazole 30(µg), ampicillin (30µg) and of ofloxacin (10µg). The tests were performed on Mueller Hinton agar and incubated at 35°C for 24h. Results were recorded by measuring the inhibition zone diameters and scored as susceptibility ranges according to the standard table (Prescott *et al* 1999; De La Rosa *et al* 1993; Anon 1988).

RESULTS

The distributions of bacterial and fungal colony counts are as shown in Table 1. None of the relaxers contained viable bacteria. Two cream samples, WMT and CET, and two toothpaste samples, CNP and HDP yielded viable counts of bacteria. Bacterial colony counts from WMT

was 221 cfu g⁻¹ and 120 cfu g⁻¹ for CET. The contaminated toothpaste samples had lower viable counts of 117 cfu g⁻¹ for CNP and 96 cfu g⁻¹ for HDP.

Analyte samples were contaminated more heavily with fungi than with bacteria with four, out of the six relaxer samples showing some level of fungal contamination. No viable fungal growth was recovered from the HDP toothpaste (Table 2).

The pH of the samples are shown in Table 1, with the relaxers being alkaline (pH range of between 10.2 to 12.2); the toothpastes slightly acidic and the creams slightly acidic to near neutral.

The genera of bacteria isolated from the cosmetic samples are *Bacillus*, *Lactobacillus*, *Pseudomonas* and *Staphylococcus*. Fungi isolated are species of *Mucor*, *Geotrichum*, *Curvularia*, *Trichophyton*, *Aspergillus*, *Fusarium* and *Cladosporium* (Table 2).

Table 3 shows the antibiotic resistance pattern of the bacteria isolated; 33% of the *S. aureus* were resistant to lincomycin and 8% to erythromycin, streptomycin and ampiclox. *Pseudomonas* sp presented the highest resistance of 55% to ampicillin. The isolates were most susceptible to co-trimoxazole with only 6% of the *Pseudomonas* sp resistant to it.

Table 1: Range of pH and Distribution of Microbial Contaminants in Analyte Products.

Products	No. tested	No. and Percentage		Microbial counts (cfu/g)		
		pH Range	Bacteria	Fungi	Bacteria	Fungi
Relaxers	18	10.2 – 12.2	0(0.0)	6(33.3)	-	67-216
Creams	18	4.2 – 8.2	3(16.7)	8(44.4)	120 – 221	73-200
Tooth pastes	12	5.2 – 7.1	3(25.0)	3(25.0)	96-117	91-87

Table 2: Distribution of Microbial Contaminants in Analyte samples.

Product	Name	Contaminating microorganism		
		Bacteria	Fungi	
Relaxers:	VGR	-	<i>Curvularia</i> sp.	
	VNR	-	-	
	PTR	-	<i>Mucor</i> sp.	
	DLR	-	<i>Geotrichum</i> sp.	
	PNR	-	-	<i>Mucor</i> sp.
		-	-	<i>Geotrichum</i> sp.
		-	-	<i>Trichophyton</i> sp.
		-	-	<i>Fusarium</i> sp.
		-	-	<i>Cladosporium</i> sp.
		-	-	-
Creams:	TPT	-	-	
	MVT	-	<i>Cladosporium</i> sp.	
	WMT	<i>S. aureus</i>	-	-
		<i>Bacillus</i> spp. <i>Lactobacillus</i> spp.	-	<i>Mucor</i> sp.

	SCT	-	<i>Mucor sp.</i>
	CET	<i>Lactobacillus spp.</i>	<i>Mucor sp.</i>
		<i>Bacillus spp.</i>	<i>Aspergillus sp.</i>
	SK?	-	<i>Mucor sp.</i>
		<i>Trichophyton sp.</i>	<i>Geotrichum sp.</i>
			<i>Cladosporium sp.</i>
Tooth pastes:	HDP	<i>S. epidermidis</i> <i>Pseudomonas sp.</i>	
	CUP	-	<i>Aspergillus sp.</i> <i>Cladosporium sp.</i>
	CNP	<i>S. epidermidis</i> <i>Bacillus spp.</i>	<i>Cladosporium sp.</i>
	DBP	-	<i>Geotrichum sp.</i>

Table 3: Antimicrobial resistance pattern of bacterial isolates

	(cfu)	Bacteria No of isolate Number (and percentage) of resistant isolate									
		L	GM	Ra	E	C	S	AX	TXS	Am	Ta-
<i>S. aureus</i>	12	4(33)	3(25)	-	(8)	-	1(8)	1(8)	-	4(33)	-
<i>S. epidermidis</i>	43	8(42)	21(49)	6(14)	12(28)	-	3(7)	7(16)	-	6(14)	-
<i>Bacillus spp.</i>	57	13(23)	9(33)	10(18)	8(14)	2(4)	4(7)	10(18)	-	10(18)	3(5)
<i>Lactobacillus spp.</i>	29	12(41)	14(48)	-	9(31)	-	1(3)	4(14)	-	6(21)	4(14)
<i>Pseudomonas spp</i>	18	7(39)	7(39)	6(33)	8(44)	6(33)	8(44)	4(22)	1(6)	10(56)	5(28)

L, Lincomycin.; Gm, gentamicin; Ra, rifampicin; E, erythromycin; C, chloramphenicol; S, streptomycin; Ax, ampiclox; Txs, co-trimoxazole; Ta, ofloxacin; Am, ampicillin.

DISCUSSION

This survey has demonstrated that a diverse range of cosmetic products and toiletry preparations contained fewer than 300 viable bacterial colony forming units per gram of product and that most of the cosmetics tested were bacteriologically wholesome as less than 20% contained viable bacteria. This is in consonance with earlier reports (Jarvis et al 1974; Baird, 1977) that over 50% of most cosmetic items are essentially "sterile". This may be as a result of improved manufacturing techniques and use of effective preservative systems that will ideally produce and maintain an adequately preserved product (Palmieri, 1983). The pH of the products may also be a contributing factor to the bacteriological wholesomeness of most of the products. Vegetative bacteria are unlikely to survive for significant periods in the alkaline pH of the hair relaxers. One can therefore say that bacteriologically most of the analysed products are safe for use since, for the most part, cosmetic preparations are for topical applications.

However, the presence of such pathogenic and potentially pathogenic organisms as *S. aureus*, and *Pseudomonas sp* is worrisome. This concern becomes more serious with the *Pseudomonas sp* showing 55% and 44% resistance to ampicillin and streptomycin respectively and the *S. aureus* species showing 33% resistance to both lincomycin and ampicillin and 25% resistance to gentamicin. Also the

Lactobacillus sp showed 48% and 41% resistance to gentamicin and lincomycin respectively. The presence of these multidrug resistant organisms in cosmetics may lead to the development of clinical infections in users of such products. Thus it will be right to assume that no hazard exists with the topical use of these contaminated products especially those contaminated with such fungi as *Trichophyton spp.* As has been reported earlier (Dixon and Dolan, 1981) *Pseudomonas* contamination in a variety of products over the past 20 years or more in industry has caused serious production problems and continues to represent a spoilage and potential health hazard.

Also of health concern is presence of viable fungi in more than 75% of the products tested. Most of the analyte products contained one type of fungus or the other with *Mucor sp* being the most frequent in occurrence. This may have been the result of exposure of these products to fungal spores which are usually airborne and have the ability to survive various pH ranges and in most of the commonly used cosmetic preservatives especially the parabens. Earlier report (Berke and Rosen, 1980), has shown that the parabens alone provide inadequate preservation for many creams and lotions. The clinical implication of the presence of these fungi in cosmetics and toiletries stems from the fact that some of them are opportunistic superficial and systemic pathogens and can take advantage of being introduced on and / or into the body. For example numerous species of *Aspergillus* and *Fusarium* have been implicated (Evanš and Richardson, 1989) in otomycosis and mycotic keratitis. The presence of *Pseudomonas spp* and

S. epidermidis in tooth pastes may not pose a serious public health problem since members of these bacteria genera have not been implicated in periodontal diseases (Prescott et al 1999). However such contaminations should be guarded against in view of the fact that periodontitis is considered a multifactorial infectious disease (Van Winkelhoff, 2003) in which many bacteria species play opportunistic but essential roles.

It is a cause for concern that cosmetics and toiletries meant to protect and preserve the skin (Otte, et al 2005), the teeth (Ponduri et al 2005), and the body generally can act as vehicles for introducing opportunistic pathogens to the body.

It is recommended here as earlier (Dixon and Dolan 1981) that emphasis should always be placed on good hygienic manufacturing practice along with careful monitoring of raw materials and finished products. An effective preservative system should be included essentially in order to prevent multiplication of the minimal number of organisms which may still occur in a well made product and to control subsequent contamination during use.

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