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Impact of Some Hair Cream Compositions on the Flora Density of Human Scalp

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The study assessed the effect of some brands of hair creams coded MBB and SDR on the flora density of the human scalp in parts of Port Harcourt. Twenty volunteered persons subjected their scalp aseptically; for swap collection before and after application of the coded hair creams. Standard microbiological procedure was adopted to culture the scalp samples on prepared sterile nutrient agar, mannitol salt agar, MacConkey agar and Sabouraud destrose agar media. All the inoculated culture media plates were incubated in their respective temperature requirements and after which colonies that developed were counted. The result showed heterotrophic bacteria counts of 9.8 x 103 and 9.3 x 103CFU/ml for before and after application of SDR hair cream, while counts of 1.05 x 104 and 1.07 x 104CFU/ml were obtained from MBB hair volunteers before and after application respectively. Similarly, a Staphylococcal count of 4.5 x 103 and 3.7 x 103CFU/ml were obtained before and after application of SDR hair cream, while counts of 6.2 x 103 and 5 x 102 CFU/ml were obtained from MBB hair before and after application respectively. A total of 53 isolates were recovered from which two bacteria genera namely: Staphylococcus epidermidis,

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Staphylococcus aureus and a fungi genera named Candida krusei were identified. Staphylococcus aureus and Staphylococcus epidermidis were noted with a 12% and 56% occurrence respectively, in sample SDR volunteers. MBB cream volunteers, recovered 14.8 and 7.4 % frequency occurrence for Staphylococcus epidermidis and Staphylococcus aureus respectively. Thus, a negative effect of hair cream SDR on the human scalp was observed. The SDR hair cream significantly, reduced the Staphylococcal normal flora of the scalp, which can lead to the development of opportunistic infection. The study recommends that users of SDR cream to be mindful in application due to creams composition.

Keywords: Human scalp; hair cream composition; microbial flora density; infection.

1. INTRODUCTION

Hair cream supplements vary in ingredients and compositions, the composition however, might interfere or alter the scalp microbiome at that given time of application [1]. Some hair supplements contain disrupting ingredients or compounds that alter the existence of microbes, and thus lead to progressive hair losses, hair fall and scalp exposure [1]. According to Townsend et al. [1] sometimes when these supplements are applied, the scalp is confronted with several diseases such as dandruff, atopic dermatitis, ance, eczema amongst others. The scalp is known to provide the first line of defense against pathogens and also shields the hair follicle and scalp from hair damage, thus resulting to a stronger scalp barrier and healthier hair [2]. Clavaud et al. [2] stated that the scalp is particularly unique, due to the high volume of vitamin rich sebum produced in the sebaceous glands whereof the scalp gives rise to cutaneous microbes that are not found anywhere else in the body [3]. The scalp microbiome is vital for keeping the scalp and hair healthy, like the skin microbiome [2]. The alteration of the scalp microbiome may trigger the pathogenic properties of the commensals which notably results to scalp diseases [3]. Ance, a typical scalp disease is remarkably, caused by *Cutibacteria*, with virulent factors such as free fatty acids and porphyrins [4]. Similarly, *Streptococcus epidermidis,* a commensal of the human scalp, if altered could causes severe skin condition (atopic dermatitis) [5]. Hair cream helps strengthen scalp barriers and prevent hair loss [6]. According to Abraham et al. [6] application of hair cream on the scalp offers so many benefits, amongst which are: hair nutrition, enhancement of hair growth and strengthening of the scalp. Other benefits include: the creams ability to heal wounds, its anti-itching, anti-inflammatory and anti-oxidant properties. Creams are also noted to prevent dryness and hair loss [1]. The application of hair cream supplement for good hair growth, beauty and aesthetic effect cannot be overemphasized. Some hair cream products with active ingredients such as parabens are likely to reduce the population of flora present on the human scalp [7]. However, some of the ingredient are useful in preventing hair loss and hence, satisfy the users admiration [6]. Different cosmetic industries have come up with diverse chemicals used for the production of hair cream products, in the absence of organic ingredients [7]. Notable ingredients include; noggin, SCF, Treipeptide, Biotinoyl, Panthenol, Biotin, Copper Tripeptide-1, sulfates, mineral oil, parabens, denatured alcohols, synthetic fragrance, formaldehyde, coal tar, phthalales, para-pheny [1]. Reis and Dias [8] noted some trade mark names such as Exosome Hair fill, Foliprime, Human Nature, Sakuru Shampoo and many others. The study therefore, seeked to consider some commonly, used hair care products displayed for public use for hair beautification vis-à-vis effect on the normal flora of the human scalp, in which users harbor opportunistic pathogen and hence opportunistic infection resulting from flora disorder. Hence, the study aimed at assessing the impact of hair cream compositions on human scalp microbial flora, with specific interest on heterotrophs due to their demand for already made nutrient [9]. Thus standard microbiological procedures were adopted.

2. MATERIALS AND METHODS

2.1 Sample Collection

Two (2) commonly used hair care products named Olive Shea Butter Hair Mayonnaise and Soul Mate Hair Care Cream that are sold in the study area (Port Harcourt) were purchased from retailers labelled with codes MBB and SDR for the purpose of the study. Olive Shea Butter Hair Mayonnaise was labelled sample MBB while Soul Mate Hair Care Cream was labelled sample SDR. The unique difference in active ingredients

of the creams compositions are disclosed as follows,: Sample code MBB contained a mineral oil, petroleum jelly, fragrance, shea butter, paraffin wax, color, olive oil, peppermint oil, vitamin E preservative (50% by weight of unsaturated fatty acids and about 50% by weight of saturated fatty acids) while sample Code SDR, contained an ingredient compositions of petroleum jelly, lanolin, mineral oil, methanol, chamomile herbal extract, bergamot oil, peppermint oil, yellow, fragrance. Consequently, the ingredients were not quantified as seen on user's manual/leaflet. Both hair cream samples were purchased and taken to the Biology laboratory of Ignatius Ajuru University of Education, Port Harcourt, Nigeria.

2.2 Experimental Design

Experimental design involved taken swab samples of 20 persons who volunteered and subjected their head scalp for the investigation. Volunteers with long or sprayed hair were excluded from participating. Thus, only volunteers who were on low cut were considered. The scalp samples were collected in an aseptic condition with the use of sterile glove and a pre-sterilized disposable swab. The samples were collected before and after application of the creams within one hour (1hr). Sample collection involved taken swab and introducing it into a sterile prepared normal saline, allowed to stand for 8 hours for resuscitation of viable microbial cells. Heterotrophs, coliforms and fungi microbes were sorted with an exception for *Cutibacterium* a non-pathogenic with low virulence [10].

2.3 Preparation of Samples

The preparation of the sample entailed carrying out serial dilution as carried by Clavaud et al. [2]. Sterile liquid called the diluents, composed of distilled water (1 liters) and Sodium Chloride (8.5g) were compounded and dispensed (9ml) into test tubes for sterilization. After sterilization the pre-disposable swab stick was streaked on the head of volunteered participants and thereafter the swab stick introduced inside the sterile 9ml diluent. In preparing the sample for investigation of the microbial load and identity, one (1) ml from the 24hrs investigative swab samples was aseptically transferred into the test tube in series of 10^{-1} dilution, followed by 10^{-1} dilution and thereafter a dilution factor of 10^{-3} was adopted for the study. The series of dilution, aimed at reducing the population of viable cells in the samples.

2.4 Preparation of Media

The agar media: (i) Nutrient agar, a general-purpose agar for growth of wide varieties of non-fastidious microorganisms, (ii) Mannitol salt agar, for growth and selective isolation of *Staphylococcal* organisms, (iii) MacConkey agar, for development and differentiation of lactose fermenting and non-lactose fermenting enteric bacilli and (iv) Sabouraud Dextrose agar, for isolation of fungi. All agars needed for the study were prepared as directed by the manufacturer as stated also by Basu et al. [11].

2.5 Enumeration / Isolation of the Microbes

The spread plating technique as carried out by Amadi-Ikpa and Awari [12] was adopted in enumerating the microbes present in the samples. The technique involved inoculating an inoculum of 0.1 volume from the 10^{-3} diluted sample using a sterile one (1) ml pipette on the various prepared media accordingly. Thereafter, the inoculum was spread using a sterile jockey stick and the media incubated for 24 hours at 37^{o}C for bacteria growth, and for fungal growth the Sabouraud Dextrose media were incubated under room temperature. Growth after incubation showed colonies which were counted and noted colony forming unit per mill [13].

2.6 Morphological / Colonial Characterization of the Isolates

Macroscopic description of the colony appearance vis-à-vis color, size, elevation, edge, opacity and shape of the bacterial and fungal colonies on growth media plates were considered. Furthermore, characterization also adopted Gram procedure to determine the isolates Gram reaction as carried out by Basu et al. [11]. The procedure involved heat fixing the bacteria colony on a clean glass slide. Staining with crystal-violet, iodine, ethanol and safranin was then followed accordingly. Gram positive bacteria showed purple color under a light microscope while Gram negative bacteria showed pink color. The morphogenesis of the isolated fungi was determined with wet preparations of the sub-cultured fungi placed on a clean greased glass slide. And with the aid of microscope the isolate was viewed. Macroscopic examination of the isolates was also considered and observations reported as described by Watanabe [14]. Fungal features observed were compared with fungal sample from a pictorial atlas [14].

2.7 Biochemical Identification of the Bacteria

Biochemical test to identify the isolates involved the use of industrial preparations of the following reagents, Oxidase, Methyl Red, Voges-Proskauer, Citrate, Glucose, Sucrose Lactose, Catalase. Other test includes: Coagulase test, Motility test, Capsule staining test and Indole test. All industrial preparations of the reagents were prepared for use as adopted by Coveny [15].

2.8 Methyl Red (MR)/ Voges-Proskauer (VP) Test

The test involved introducing the test bacteria into 10ml sterile MR/VP broth medium prepared according to manufacturer's instructions, followed by incubation of the medium. The broth was the shared into two parts (5ml) each after incubation. To one part noted as methyl red part while the other part Voges-Proskauer. To the part with methyl red, 5-6 drops of methyl red reagent was added and to the part with Voges-Proskauer, 0.6ml (6 drops) of 5% a – naphthol and 0.2ml (2 drops) of 40% KOH were added. Development of bright red coloration is indicative of positive methyl red while the reverse is negative. For the Voges-Proskauer a change in the medium indicate positive result while an absence of change in the medium indicated a negative result.

2.9 Citrate Test

The test determined the ability of the bacteria to utilize Sodium Citrate as its sole source carbon and inorganic ammonium salt as its only source of nitrogen. Simmon Citrate agar was prepared and with the aid of sterile wire loop, the test bacteria were introduced into the prepared sterile medium. The medium was then incubated at 37^0C for 24hrs. Change in color from green to blue indicated a positive result whereas a no change in color indicated a negative result.

2.10 Sugar Fermentation Test

The test evaluated the ability of the bacteria to utilize sugar (Lactose, Sucrose and Glucose) to produce acid and gas. Peptone broth (1%) incorporated with 1% sugar was used to constitute the sugar broth test. An indicator was added to the sugar medium with Durham tube added in the tube in an inverted position. After sterilization, a loopful of the bacteria was introduced into the test tubes and then incubated at 35 -37^0C for 24 - 48 hrs. Change in color from purple to yellow and gas production indicated a positive sugar fermentation test, while no change in color depicted no sugar utilization [12].

2.11 Indole Test

The test determined the ability of the bacteria to split the amino acid tryptophan to form pyruvic acid, ammonia and indole using the enzyme Tryptophanase. The test bacteria were inoculated into sterile peptone water medium and incubated at 37^0C for 48hrs. thereafter, 0.3 – 0.5 ml of Kovac's reagent was added using a Pasteur's pipette. Appearance of red ring layer on the medium indicated a positive Indole test while development of a yellow ring indicated a negative result [12].

2.12 Catalase Test

The test involved placing the unidentified bacteria into a glass slide followed by introducing 3% of the reagent into the test slide. Production of effervescence by the isolate was determined by the use of Catalase test, where the un-identify isolate breaks down Hydrogen Peroxide into water and oxygen, thus signifies positive reaction and the reverse, negative reaction [12].

2.13 Coagulase Test

A clotting inference after few seconds' introduction of the human plasma into the slide containing the test bacterium is reported to indicated positive Coagulase while an absence indicated a coagulase negative bacterium as documented by Amadi-Ikpa and Awari [12]. The test involved placing the test bacteria on a clean glass slide with two to three drops of saline and a human plasma, and the substance mixed thoroughly and allowed to clot.

2.14 Motility Test

This test involved the preparation of a semi solid Nutrient agar medium and the test organism inoculated into the media by stabbing using a sterile straight wire. Thereafter, the growth media was incubated at 37^0C for 24 - 48 hrs. Growth in diffuse form from the line of stab into the medium indicated a positive result for the presence of flagella, whereas growth only along the line of stab indicated a negative result for the absence of flagella [12].

2.15 Capsule Staining Test

The test procedure involved the addition of a few drops of crystal violet onto the test bacteria. The test bacteria were placed on a clean microscopic slide to receive the reagent. Having introduced the reagent on the bacteria, the component was stirred and viewed under a light microscope. A light blue appearance signified encapsulated cell, while the reverse signified an un-encapsulated cell [12].

2.16 Data Analysis

Data generated were analyzed using T-test statistical tool (analysis of variance) was adopted to test for significant differences between the variables with regard to the hypotheses. The P-value was used as decision rule for accepting or rejecting the null hypothesis and the level of significance set at 0.05 as adopted by Nester et al. [16].

3. RESULTS

3.1 Enumeration of Microbial Load

Table 1 showed the counts of the microbial load on the scalp of volunteers. Counts of total Staphylococcal, coliforms, heterotrophic bacteria and fungi were detected on scalp before and after application of the various hair supplements. The counts varied between the two sessions. Table 1 showed heterotrophic bacteria counts of 9.8×10^3 and 9.3×10^3CFU/ml for before and after application of SDR hair cream, Thus, showing no significate difference at P>0.05, while counts of 1.05×10^4 and 1.07×10^4CFU/ml were obtained from MBB hair volunteers before and after application respectively, with no significant difference. In a similar count, Staphylococcal count of 4.5×10^3 and 3.7×10^3CFU/ml were obtained before and after application of SDR hair cream showing no significant difference, while counts of 6.2×10^3 and 5×10^2CFU/ml were obtained from MBB hair before and after application respectively, statistically, showing a significant difference. Coliforms were not observed before and after application of the hair cream. No counts were obtained for heterotrophic fungal before and after application of the hair creams.

3.2 Characterization of Bacterial Isolates

Table 2 showed the biochemical reactions of some bacterial isolates recovered from the scalp observed in the study. The study recovered two bacteria genera namely: *Staphylococcus epidermidis*, and *Staphylococcus aureus.* Phenotypic feature showed the color of colonies on culture plates with probable bacteria as *Staphylococcus aureus* and *Staphylococcus epidermidis*. Furthermore, phenotypic feature of the isolates/colonies based on size, shape and surface edges revealed that both isolates had common features of small sizes, circular shapes, smooth surfaces, low elevation, curved edges and opaque.

Also, Table 3 showed the biochemical reaction as characterized, Gram positive reaction was observed for both bacteria, distinguished with coagulase and citrate properties which S*taphylococcus aureus* possesses.

Table 1. Microbial load

Bacteria	Sample MBB (cfu/ml)			Sample SDR (cfu/ml)		
	Before	After	T-test	Before	After	T-test
Staphylococcal	4.5×10^3	3.7×10^3	P>0.05	6.2×10^3	5×10^2	P<0.05
Fecal coliform	0	0	-	0	0	-
Coliform	0	0	-	0	0	-
Heterotrophic Bacteria	9.8×10^3	9.3×10^3	P>0.05	1.05×10^4	1.07×10^4	P>0.05
Heterotrophic fungi	0	0	0	0	0	-

Key ---cfu/ml= coliform forming unit per mill

Table 2. Morphological characterization of bacterial isolates

Iso	Size	Shape	Surface	Ele.	Edge	Opacity	Colour
1	Tiny	Round	Smooth	High	Curve	Opaque	Grey
2	Large	Round	Smooth	Low	Curve	Opaque	Yellow

Key; Iso= Isolate, Ele.= Elevation

Table 3. Biochemical characterization of bacterial isolates

G. Sta	MR	VP	CT	IN	CA	OX	CO	SU	GL	LA	MO	CP	Probable Bacteria
+	+	-	-	-	+	-	-	+	+	+	-	+	*S. epidedimis*
-	+	+	+	-	+	-	+	+	+	+	-	+	*S. aureus*

Key; G.sta= Gram Stain, MR= Methyl Red, VP= Voges Proskauer, CT= Citrate, IN= Indole, CA= Catalase, OX=Oxidase, CO= Coagulase, SU= Sucrose, GL=Glucose, LA= Lactose, MO=Motility, CP= capsule, S = Staphylococcus, + = Positive, - = Negative

Table 4. Macroscopic characterization of the fungi isolate

Isolate	Structural description				Identification
	Color	Size	Growth rate	Texture	
1	Milky	Small	Fast	Shiny	Yeast Cell

Table 5. Frequency of occurrence of the microbial isolates

S/no	Microbial Isolates	MBB		SDR	
		Frequency of Occurrence	Percentage Composition (%)	Frequency of Occurrence	Percentage Composition (%)
1	*Staphylococcus aureus*	9	33	10	38.3
2	*Staphylococcus epidermidis*	14	52	11	42.3
3	*Candi krusei*	4	15	5	19.2

3.3 Macroscopic Characterization of the Isolated Fungi

Table 4, showed the macroscopic characterization of the fungal isolate. The study recovered a fungal present in the human scalp before and after application of the hair-care products. The fungi *candida krusei* was identified whitish, in color with a shiny textured appearance.

3.4 Frequency of Occurrence of the Microbes Isolates

Table 5: below showed the microbial prevalence in the human scalp from both sample supplements on volunteers. High prevalence of the isolates were noted in sample supplement MBB as against the SDR. The percentage occurrence of bacteria in sample MBB volunteers was 12.5% for *Staphylococcus aureus* and 56% for *Staphylococcus epidermidis.* For volunteers with application of sample SDR, the percentage occurrence of *Staphylococcus epidermidis* and *Staphylococcus aureus* where 14.8% and 7.4% respectively. The fungal occurrence where noted 15 and 19.2% for supplements MBB and SDR respectively.

4. DISCUSSION

The insignificant difference in counts of heterotrophic bacteria may have shown no pathogenic conditions observed with the application of the hair product, however the cream may have increased the number of heterotrophic bacteria count since the heterotrophs depend solely on organic nutrients which the hair cream provides [17]. The study showed heterotrophs on the scalp must have consumed some of the ingredients which are mostly, organic carbon. These ingredients served as a food source for the microbes. Heterotrophs presence in human may promote opportunistic infections nevertheless, heterotrophic bacteria have not been linked to pathogenic conditions [18]. The absence of coliform counts as reported in this study, has also been reported by Kerk et al. [19]. However, if coliforms were present, there presence would have indicated insufficient sanitary condition [19]. The absence of coliform as noted further showed no incidence of potential disease. Basically, in another instance, the presence of coliform may suggest the presence of lice. Lice on human scalp are associated with excretion, where they defecate feces and urine on human scalp [17]. The insignificant counts of

Staphylococcal with the application of sample MBB hair product is normal, however the insignificant difference observed in the application of sample SDR hair product could be a threat to *Staphylococcal* population and hence, result to Staphylococcal infection even at the decreased load [20]. Morphological and biochemical identification of the isolates, *Staphylococcus aureus* and *Staphylococcus epidermidis* which showed high percentage frequency is stable as reported in this study. Kerk [19] identified *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeriginosa* and *Escherichia coli* on human hair shafts. Thus, the presence of *Staphylococcus aureus* on the human hair before and after application of the hair products MBB and SDR showed if altered, may cause minor skin infection. *Staphylococcus aureus* is a normal flora of the skin, and its low concentrations may still cause infection if open wounds are spotted on the scalp. With regards to heterotrophic fungi counts, even as insignificant in this study, small concentrations have the ability to cause diseases in human and animal [17]. The identified presence of fungi is attributed to the scalp humid condition. With the fungal presence the continuous application of hair cream products may initiate dandruff. Therefore, the study identified *Candida krusei* as the sole fungal present before and after application of the hair products or supplement [21].

5. CONCLUSION

The study demonstrated that the sample SDR has a significant effect on the microbial flora of the human scalp, specifically the counts of heterotrophic bacteria which were reduced after hair application. These reductions in heterotrophs and Staphylococcal open up possibilities for an opportunistic infection, in a broken human scalp at the time of application or on a low immune user. Furthermore, the study pointed out that the composing ingredients in SDR cream have more organic ingredients than the MBB product that affected the proliferation of the human scalp flora. The MBB product was more favorable to the scalp microbiome and therefore recommended for hair cream users. Basically the study was limited to the SDR and MBB hair care products due to their availability in the study area, however in some other studies, additional hair products could be explored.

6. RECOMMENDATION

The study advice that users of SDR cream compositions should avoid the use of such cream or reduce the level of application.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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