

Research Article

Microbiological evaluation of cosmetics products sourced in Aba city, Nigeria

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ABSTRACT

Background: Cosmetics are external preparations normally applied to human body parts mainly for beautifying, cleansing and protecting. These products are basically non-sterile but must be completely free of high-virulence microbial pathogens. Production of stable cosmetics products require integrated quality management system which consists of quality raw material, proper product formulation, hygienic design of production facilities, good production hygiene process, packaging containers and a validated preservative system. Inadequately preserved products can provide conducive environments for microorganisms especially in the tropical region. The objective of this study is to assess the microbial quality of some selected brands of cosmetics produced in the country and sold within the commercial city of Aba, Abia state of Nigeria thus to note the health hazards consumers are exposed to.

Methods: Twenty brands of commercially available Cosmetic products manufactured in Nigeria were evaluated for their microbial quality using standard procedures.

Results: There was no viable bacterial growth in 40% of the samples tested and no yeast growth in 65% of the samples tested. 35% of the products indicated < 300 CFU /g of samples tested. The predominant bacterial isolates were *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Preservative efficacy tests carried out on the products using *Pseudomonas aeruginosa* ATCC 9027 showed only 30% of the products tested were adequately preserved.

Conclusions: The poor microbiological quality of the preparations investigated can be attributed to either the formulation of these brands or environmental conditions during the manufacturing process. It is hoped that the training of the personnel that handle these processes will improve cosmetic products quality in Nigeria.

Keywords: Cosmetics, Preservative *Pseudomonas aeruginosa*, *Candida albican*

INTRODUCTION

Cosmetics are external preparations normally applied to human body parts mainly for beautifying, cleansing and protecting.²⁵ These products are formulated from an array of chemicals in the presence of plentiful amount of water and mostly exhibit a near neutrality pH.² Cosmetic products are basically non sterile but must be completely free of high-virulence microbial pathogens. The total number of aerobic microorganisms per gram must be at minimal stipulated standard by various authorities in any

country, for instance, International Standard Organisation (ISO), Standard Organisation of Nigeria (SON) and National Agency for Food and Drug Administration and Control (NAFDAC) as is the case in Nigeria. Production of stable cosmetics requires an integrated quality management system which consists of quality raw material, proper product formulation, hygienic design of production facilities, good production hygiene process, packaging containers and a validated preservative system.^{13,27} To achieve this, the preservatives are optimised by antimicrobial stabilisation and its synergistic effects in cosmetic products. Therefore, unless adequately

preserved, these products can provide conducive environment for microorganisms especially in the tropical region.

It can equally be liable to microbial contaminations either during the course of transportation, storage of finished goods or during use by the consumers which may lead to spoilage.¹⁵ These contaminants could be pathogens, opportunistic pathogens or saprophytes which may in turn result in economic loss and infection on the body.^{2, 4, 5} Since 1960s, opportunist organisms, such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Pseudomonas sp.*, *Serratia sp.* and *Enterobacter sp.*, have been isolated from cosmetic products to a certain level.¹⁶ A situation where these cosmetic products are heavily contaminated with pathogenic organisms, could lead to biodegradation of the product and risk of infection to consumers.⁹ This spoilage usually results to alteration in organoleptic properties of cosmetic products which may bring about colour, odour changes and biodegradation of the active component of such preparations. The products are therefore stored at ambient temperature particularly in tropical regions. It is not acceptable that the following potentially pathogenic microorganisms *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *E.coli* and other members of *Enterobacteriaceae* are present in cosmetics product.¹³

In Nigeria, guidelines on Good manufacturing Practice (GMP) of Cosmetic have been drawn by microbiology technical committee of SON to guide other regulatory agencies in the country such as NAFDAC and other stakeholders like the manufacturing companies to cover various quality aspects of cosmetics. These cover Standard operating procedures (SOP) for production, documentation, distinctive cleaning procedures, chemical, microbiological control of raw materials, bulk and finished goods. Others include packaging materials, personnel, equipment, and storage areas. Microbial contamination of cosmetic products has persisted in commercially sourced products in many developing countries despite both Local and International guidelines.^{7,24} However, developed countries have moved a step further after the implementation of GMP regulations by recalling products found to be outside the microbiological limits.^{22,31}

The objective of this study is to assess the microbial quality of some selected brands of cosmetics produced in the country and sold within the commercial city of Aba, Abia state of Nigeria thus to note the health hazards consumers are exposed to.

METHODS

Sampling

Twenty samples representing 5 different categories of cosmetic products comprising of 6 soap, 5 body cream, 4 hair cream, 2 Roll-on and 3 powder samples with

appropriate manufacturing and expiry dates were collected from different supermarkets and medicine stores in Aba, Abia state, Nigeria. All samples were tested to assess their bacterial and fungal load as well as the presence of pathogenic organisms using standard microbiological and biochemical methods.^{3,19,23}

Media used

Tryptic Soy Agar (TSA), Sabouraud Dextrose Agar (SDA), Mannitol Salt Agar (MSA), MacConkey Agar, Eosin Methylene Blue Agar (EMB), Cetrimide Agar and Brilliant Green Phenol Red Lactose Sucrose Agar (BGPR) and Potato Dextrose Agar (PDA) were used for microbial isolation and identification.

Viable microbial count

Determination of total microbial viable count for each sample was carried out using one gram of the products respectively. Each sample was dispersed into separate sterilized phosphate buffer solutions containing 0.5% Polysorbate 80 which served as preservative neutralizer and 10-fold serial dilutions were made under aseptic conditions. Pour plate technique was used on a 1-mL aliquot taken from the last dilution into a sterile petri dish, 15ml of sterile TSA poured and properly mixed with the test sample in the petri dish. The solidified agar plates were incubated at 37 °C for 48hr. Yeasts were cultured as described for bacteria using SDA. Fungi was also isolated on SDA supplemented with chloramphenicol to inhibit bacterial growth (Oxoid) and incubated at 27°C for 7days. All the experiments were carried out in triplicates. After incubation, the number of colonies were counted using colony counter to estimate the total viable colonies growing on each plate for bacteria and Yeast then the mean of three plates was taken. A laboratory control count was performed using control blank (without product sample). The test sample was considered contaminated in a situation where the colony count exceeded 300. Plates with colonies of 30-300 were selected. The microbial viable count per millilitre of sample dilution is the colony count multiplied by the appropriate dilution factor (10^{-2}).

Microbial identification

Identification of all bacterial isolates were determined based on the colony formation, Gram stain reaction and biochemical tests, as described by USFDA manual online 2001 while fungal isolates were based on both macroscopic and microscopic appearance as described by Larone.^{11,20,25}

However, further examinations on the organisms were carried out using selective media for identification as described by Urmi et al.^{26,30} Mannitol Salt Agar, MacConkey Agar, EMB, Cetrimide Agar and BGPR were used for identification of *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas*

aeruginosa and *Salmonella* respectively. All bacterial plates were incubated for 24 hours at 37°C in aerobic conditions while PDA plates for fungi identification were incubated at 28 ± 2°C and observed daily for 7 days.

Preservative effectiveness testing

Preservative effectiveness testing was described through the method reported by Campana et al.^{8,12} The antimicrobial preservative efficacy of cosmetic products was investigated using *P. aeruginosa* ATCC 9027 as recommended by USP XXVI for the evaluation of antimicrobial protection of cosmetic products. The test organism was grown on TSA and harvested with sterile phosphate buffer, pH 7. The absorbance of the test organism suspension containing 1×10^8 cfu/ml was determined using spectrophotometer at 625 nm wavelength. Fifty grams of each product sample was weighed aseptically into respective sterilized conical flask and each inoculated with 0.1 ml of test inoculum suspension. The respective samples were properly mixed until a homogeneous suspension was obtained and incubated at 28°C for 28 days. Thus each initial test product contained approximately 2.0×10^5 cfu/g as at the time of experiment. Two gram of respective samples was aseptically drawn at 0, 7, 14, 21, and 28th day into separate neutralizing medium and subsequently plated for viable count as described above. Un-inoculated sample of each product was used as control. The product was considered as adequately preserved when 99.9% reduction of the initial inoculum count was obtained on the 7th day of incubation and remained with no increase as the incubation period progressed until the final day of the experiment.^{6,28}

RESULTS

The result table represents total viable counts for bacteria and yeast from different cosmetic products. The microbial evaluation of 20 cosmetic products showed that results of most Cosmetics product samples tested exhibited high bacteria count ranging between $0.2 \pm 0.03 \times 10^2$ to $7.6 \pm 0.07 \times 10^3$ of baby powder and moisturizing cream respectively. Yeast count ranged $0.5 \pm 0.08 \times 10^2$ to $6.0 \pm 0.12 \times 10^4$ for coloured powder and styling gel. 60% viable bacterial growth was observed from the samples tested while 65% tested showed no yeast growth. About 35% of the tested products were showed microbial load <300CFU/g (Table 1).

The types of contaminants associated to various cosmetics product sampled were examined. *Pseudomonas aeruginosa* was predominant among 55% of the total samples investigated. Only moisturizing cream sample showed the presence of *Proteus vulgaris*, however, both moisturizing and body butter cream samples contained *E.coli* during the microbial examination of the cosmetic products. The presence of *Salmonella sp* was only observed in powder sample. The common yeast observed during the course of this investigation was *Candida*

albican while *Aspergillus* species were observed in 50% of the total sample examined. However, there was no microbial growth from seven samples. The presence of *Microsporium audonii* was observed in only Body butter cream (Table 2).

Table 1: Total microbial viable count.

Cosmetics Brands	Total viable bacterial count	Total yeast count
Soap		
Baby Soap	$2.8 \pm 0.06 \times 10^2$	0
Beauty Soap	$3.4 \pm 0.05 \times 10^3$	0
Medicated soap	0	0
Liquid Soap	0	0
Laundry Soap	$0.8 \pm 0.16 \times 10^2$	0
Detergent	0	0
Body Creams		
Organic body cream	$2.8 \pm 0.03 \times 10^2$	$4.4 \pm 0.14 \times 10^2$
Moisturizing cream	$7.6 \pm 0.07 \times 10^3$	$2.7 \pm 0.04 \times 10^2$
Complexion cream	0	0
Body Butter creams	$1.8 \pm 0.17 \times 10^3$	$2.4 \pm 0.07 \times 10^3$
Shaving cream	$2.0 \pm 0.17 \times 10^2$	0
Hair Creams		
Darkening Hair cream	0	0
Anti dandruff hair cream	0	0
Hair food	0	$1.2 \pm 0.03 \times 10^3$
Hair Styling gel	$0.2 \pm 0.04 \times 10^2$	$6.0 \pm 0.12 \times 10^4$
Roll-on		
Deodorants	$4.3 \pm 0.04 \times 10^2$	$5.8 \pm 0.21 \times 10^3$
Antiperspirant	0	0
Powders		
White Facial powder	$1.3 \pm 0.05 \times 10^2$	0
Coloured powder	$6.3 \pm 0.06 \times 10^2$	$0.5 \pm 0.08 \times 10^2$
Baby powder	$0.2 \pm 0.03 \times 10^2$	0

The preservative effectiveness test was performed on all the products samples used in this research with emphasis on those that did not show sign of contamination during the total viable count using *P. aeruginosa* ATCC 9027. This result clearly demonstrates that four out of 20 cosmetic samples passed the preservative effectiveness test. Six of these 16 products were the ones to yield the highest microbial counts with preservative activity results above 1×10^6 (Table 3).

Table 2: Types of microbial contaminants isolated from various products category.

Cosmetics Brands	Bacterial isolated	Yeast isolated	Fungi isolated
Soap			
Baby Soap	<i>P. aeruginosa</i> , <i>S. aureus</i>		
Beauty Soap	<i>P. aeruginosa</i> , <i>Bacillus sp</i>		<i>A. niger</i>
Medicated soap			
Liquid Soap			
Laundry Soap	<i>P. aeruginosae</i>		<i>A. niger</i>
Detergent			
Body Cream			
Organic body cream	<i>P. aeruginosa</i> , <i>B. subtilis</i> , <i>S. aureus</i> , <i>Enterobacter aerogenes</i>	<i>C. albican</i>	<i>A. flavus</i>
Moisturizing cream	<i>P. aeruginosa</i> <i>S. aureus</i> , <i>E. coli</i> , <i>Proteus vulgaris</i> , <i>Micrococcus spp.</i>	<i>C. albican</i>	<i>A. niger</i>
Complexion Cream			
Body Butter creams	<i>P. aeruginosa</i> , <i>S. aureus</i> , <i>E. coli</i> , <i>E. aerogenes</i> , <i>Micrococcus spp.</i>	<i>C. albican</i>	<i>A. niger</i> , <i>M. audouinii</i>
Shaving Cream	<i>S. aureus</i>		
Hair Creams			
Darkening Hair Cream			
Anti-dandruff Hair Cream			
Hair food		<i>C. albican</i>	<i>A. niger</i>
Hair styling gel	<i>P. aeruginosa</i> , <i>S. aureus</i> , <i>Micrococcus spp.</i>	<i>C. albican</i>	<i>A. niger</i> , <i>A. flavus</i>
Roll-on			
Deodorants	<i>P. aeruginosa</i> , <i>S. aureus</i>	<i>C. albican</i>	<i>A. niger</i>
Antiperspirant			
Powders			
White Facial powder	<i>Salmonella</i> , <i>P. aeruginosa</i>		
Coloured powder	<i>Salmonella</i> , <i>S. aureus</i>	<i>C. albican</i>	<i>A. niger</i>
Baby powder	<i>S. aureus</i>		<i>A. niger</i>

S. aureus- *Staphylococcus aureus*, *P. aeruginosa*-*Pseudomonas aeruginosa* *B. subtilis*- *Bacillus subtilis*, *C. albican*- *Candida albican*, *A. niger*- *Aspergillus niger*, *E. Aerogenes* - *Enterobacter aerogenes*, *E.coli*- *Escherichia coli*, *M. audouinii* - *Microsporium audouinii*

DISCUSSION

Cosmetics are not meant to be sterile as most constituents form nutrients for the growth of various micro organisms.^{18,27} However, they are not to contain pathogens and total viable count is expected to be ≤ 300 cfu/g so as not to impair skin and mucous membrane defence mechanisms.²⁷ This research was carried out to investigate the microbial load in twenty different samples of cosmetics that are produced in Nigeria and sold within the city of Aba, Abia state of Nigeria. The samples used in this research were inspected for physical appearance; all were neat and sealed. A visual check revealed that all creams or emulsions were homogeneous. The mixtures showed no separation, sedimentation neither discoloration nor growth in any of the products. Only four manufacturers indicated methyl and propyl paraben as the preservative in the body cream sample used in this research. Two soap and deodorant samples respectively indicated triclosan as preservative used for production. There was no indication of use of preservative in any of the hair product samples used probably because they are all jelly based products, also there was no indication of the presence of preservation in powder samples. All other preparations did not specify the name of the preservative.

The microbial evaluation of these products exhibited high bacteria count ranging between $0.2 \pm 0.03 \times 10^2$ to $7.6 \pm 0.07 \times 10^3$. Yeast count ranged $0.5 \pm 0.08 \times 10^2$ to $6.0 \pm 0.12 \times 10^4$ for coloured powder and styling gel (Table 1). The soap samples showed no yeast growth while all the cream samples showed presence of bacteria.

Pseudomonas aeruginosa was predominant among most samples except in 45% of the total samples investigated (Table 2). Only moisturizing cream showed the presence of *Proteus vulgaris* while both moisturizing and body butter cream samples contained *E.coli* during the microbial examination of the cosmetic products. The presence of *Salmonella sp* was only observed in powder sample. The common yeast observed during the course of this investigation was *Candida albican* while *Aspergillus* species was observed in up to 50% of the total sample examined. However, there was no microbial growth on seven samples. The presence of *E.coli* in body butter cream indicate poor hygiene condition from manufacturer and this can be controlled by sanitary processing and using appropriate and adequate preservatives. According to the European Union (EU) legislation, cosmetic products must not contain more than 1,000 CFU/g cream while *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* must not be detectable in 0.5 g of the product.²⁹ The result on table 1, indicate that about 80% Of the samples tested were within the microbial load specification of the European union standard considering the manufacturing dates on products but the presence of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* in most of the samples nullifies the sanitary conditions of the manufacturing processes of these products. The present study is in tandem with the

previous findings of Abdelaziz *et al*; who reported cosmetic creams to harbour a high number of bacteria and fungi including hazardous type such as: *Staphylococcus epidermidis* and *Micrococcus spp.*

Staphylococcus aureus was predominant and remains the most common bacterial skin pathogen associated with skin boils, impetigo, conjunctivitis, folliculitis and food poisoning.^{1,10}

Table 3: Preservative effectiveness testing.

Cosmetics brands		Days of incubation			
Soap	0	7 th	14 th	21 st	28 th
Baby Soap	1.9 ±0.03x 10 ⁵	3.6 ±0.5 x 10 ⁷	Numerous	Numerous	Numerous
Beauty Soap	2.0 ±0.15x 10 ⁵	6.8 ±0.3 x 10 ⁷	Numerous	Numerous	Numerous
Medicated soap	1.9±0.26 x 10 ⁴	0.86±0.2x 10 ³	0.82±0.2x10 ²	0.82±0.5x10 ²	0.79±0.3x10 ²
Liquid Soap	2.0±0.01 x 10 ⁵	5.0±0.8 x 10 ⁷	Numerous	Numerous	Numerous
Laundry Soap	2.1±0.04 x 10 ⁵	6.5±0.2 x 10 ⁷	4.3±0.3 x 10 ⁹	Numerous	Numerous
Detergent	2.2±0.02 x 10 ⁵	1.6±0.2 x 10 ⁴	1.5±0.2 x 10 ⁴	1.4±0.8 x 10 ³	1.4±0.3 x 10 ³
Body cream					
Organic body cream	1.9±0.05 x 10 ⁴	5.9±0.5 x 10 ⁶	Numerous	Numerous	Numerous
Moisturizing cream	1.8±0.13 x 10 ⁵	6.2±0.3 x 10 ⁸	Numerous	Numerous	Numerous
Complexion cream	2.1±0.06 x 10 ⁵	0.7±0.2 x 10 ²	0.4±0.2x10 ²	0.31±0.2x10 ²	0.22±0.3x10 ²
Body Butter creams	2.3±0.05 x 10 ⁵	6.4±0.2 x 10 ⁶	Numerous	Numerous	Numerous
Shaving cream	1.9±0.02 x 10 ⁵	4.9±0.6 x 10 ⁶	2.3±0.3x10 ⁷	1.8±0.3 x 10 ⁹	Numerous
Hair Creams					
Darkening Hair cream	2.1±0.01 x 10 ⁵	0.1±0.6 x 10 ²	0.1±0.4x10 ²	0.1±0.2 x 10 ²	Nil
Anti dandruff hair cream	1.9±0.03 x 10 ⁵	0.4±0.4 x 10 ²	0.4±0.2x10 ²	0.4±0.3 x 10 ²	0.4±0.3 x 10 ²
Hair food	2.1±0.02 x 10 ⁵	1.7±0.3 x 10 ³	1.5±0.3x10 ²	0.8±0.6x10 ²	0.7±0.3 x 10 ²
Hair Styling gel	1.8±0.03 x 10 ⁵	4.8±0.14x 10 ⁶	3.4±0.4x10 ⁸	Numerous	Numerous
Roll-on					
Deodorants	2.3±0.06 x 10 ⁵	2.9±0.06x 10 ⁷	3.7±0.3x10 ⁷	Numerous	Numerous
Antiperspirant	1.9±0.0x 10 ⁵	3.6±0.03x 10 ⁶	1.6±0.2x10 ⁸	Numerous	Numerous
Powders					
White facial powder	2.0±0.02 x 10 ⁵	4.9±0.02x 10 ⁵	Numerous	Numerous	Numerous
Coloured powder	2.3±0.04 x 10 ⁵	4.6±0.04x 10 ⁶	Numerous	Numerous	Numerous
Baby powder	1.8±0.02 x 10 ⁵	3.1±0.02x 10 ⁵	Numerous	Numerous	Numerous

The presence of *P. aeruginosa* was observed in nine products during this test. The presence of *P. aeruginosa*, in shampoo was reported to cause infections and one death after use by immunosuppressive patients.¹⁶ *P. aeruginosa*, *B. cepacia*, *S. aureus* and *Enterococcus sp.* were observed as contaminants in 24 different cosmetic products carried out on a survey on recalls of microbiologically contaminated cosmetics in Europe between 2005 to May 2008.²² The presence of *Pseudomonas sp.*, *Staphylococcus sp.*, *Bacillus sp.* and *E. coli* are in agreement with previous report by Okeke and Lamikanra.²⁴ Similarly, Hugbo *et al* identified presence of *S. aureus* and *Bacillus sp.* in previous research.¹⁷

Considering the twenty products sampled, it was observed that the preservatives in 16 products did not comply with the USP recommended antimicrobial preservative activity criteria.²⁸ Six of these 16 products were the ones to yield the highest microbial counts and

their preservative activity results could not be determined at the seventh day of examination due to colonies observed were above 1 x 10⁶. From Table 3, it was observed that the four samples that passed preservative efficacy test with 99.9% the antimicrobial activity were mostly hair products with high level of menthol and petroleum jelly as major raw materials. These may be the cause of no growth in Table 1 and 2 respectively. Similarly, the complexion cream showed no sign of viable growth after 24h of incubation in Table 1. The active ingredients which produce the toning effect in the cream could have acted in synergy with the appropriate preservative to pass antimicrobial effectiveness test. During the course of this research it was observed that information disclosed on the labels of the cosmetic containers studied did not show the type or percentage quantity of preservatives used for the manufacture of the samples tested.

The lack of information disclosure seems to be a common problem amongst Manufacturers in Nigeria because all effort made to get the type and quantity of preservative used proved abortive as the technical crew of most the companies could not divulge this information easily.

However, the repeated failure of the microbiological test replicates of most samples indicated that these brands might have suffered from inherent problems such as handling, source of raw materials, poor sanitary properties from the environment during manufacturing processes as reported earlier on.^{14,21} The reoccurring contamination in different batches of the same brand brings doubts about the effectiveness and compatibility of the preservative system with other ingredients used in formulations. Whereby the appropriate preservatives had been used, the products would have inhibited contaminants. Similarly product pH and non-compatibility of raw material such as fragrances can be about coloration in some creams. Reports have shown that chemicals such as lipids, polysaccharide, proteins, alcohol and glucoside of cosmetics can support growth of microorganisms in the cosmetics products.^{12,18} The other factors that affect stability of cosmetics products are temperature of storage, availability of O₂ and poor activity of preservative as mentioned earlier. It has been reported that the presence of *Bacillus* sp. might be responsible for unpleasant odor and spoilage of cosmetic products.¹²

CONCLUSION

Many people use cosmetics unaware of the potential dangers that can result from contamination of such products due to microbial growth and possibly result to infections. There should be mandatory regular trainings for those in Cosmetics industries organised by the regulatory agencies such as NAFDAC, and SON with a view of improving the Good manufacturing Practice. The effect of enforcing GMP regulations in Nigeria by our regulatory agencies will help improve product quality in the entire aspects of Cosmetic production.

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