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Microbiological Quality and Characterization of Potential Pathogens Associated with Selected Brands of Commercial Cosmetic Products in Nigeria

Michael Oluyemi Babalola[1*] and Mary Eze[1]

[1]Department of Microbiology, Adekunle Ajasin University, P.M.B. 001, Akungba Akoko, Ondo State, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author MOB designed the study, wrote the protocol, performed the analyses, provided the literature searches, wrote the first drafts of the manuscript, managed the analyses of the study, read and revised the drafts of the manuscript. Author ME managed the fieldwork, performed the analyses and wrote the first drafts of the manuscript. Both Authors read and approved the final manuscript.

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ABSTRACT

Aims: Seemingly innocuous commercial cosmetic products have been responsible for serious overt and covert skin infections, which were often ignored as the sources or vehicles of transmission of pathogens.
Materials and Methods: Three (3) popular brands of commercial cosmetic products, consisting of fifteen (15) units each, of Hair straighteners, "Lip Gloss" and baby lotions, were randomly purchased from retail stores in Southwest Nigeria, and the microbiological qualities were evaluated, using the Aerobic plate count technique.
Results: From the forty five (45) samples analyzed, thirty-eight (84%) were contaminated, from which a total number of seventy (70) microbial isolates belonging to eleven (11) bacteria genera (comprising 7 Gram positive rods, 27 Gram negative rods and 10 Gram positive cocci) and nine (9) fungal genera, were isolated. Of the bacteria isolates, 50% of the Gram negatives and 52.9% of the Gram positives were multidrug resistant. The bacteria and fungal diversity in the baby lotion exceeded the hair relaxer and the Lip gloss, respectively.

**Corresponding author: E-mail: mikeolubabs@gmail.com;*

Conclusion: The isolation of *Buttiauxella agrestis*, which had not been previously reported, in addition to *Enterobacter gergoviae, Pseudomonas aeruginosa, Staphylococcus aureus, Aspergillus flavus, Penicillium* spp and *Candida albicans* (designated as objectionable microorganisms in cosmetics) indicated failures in the statutory microbiological standards. As such the products could serve as vehicles for transmission of these pathogens.

Keywords: Cosmetics; babies; pathogens; Buttiauxella agrestis; Enterobacter gergoviae.

1. INTRODUCTION

The US Food, Drug and Cosmetic Act defines cosmetics as articles intended to be rubbed, sprinkled or sprayed on, introduced into, or otherwise applied to the human body or any part thereof for cleansing, beautifying, promoting attractiveness, or altering the appearance, while maintaining the structure and functions [1]. Included in this definition are products such as skin creams, lotions, perfumes, lipsticks, eye and facial make-up preparations, hair straighteners, conditioners, shampoos, permanent waves, hair colors, and deodorants. The US FDA and the EU Cosmetics Directive requires that the microbial population be low, stable and devoid of harmful organisms, particularly in products designed to be applied around the eye and other sensitive areas, or for use by babies, young children, the elderly, and the immunocompromised [2]. While no mandatory limits are given for microorganisms, industry guidance recommends a Total Viable Counts (TVC) of <100 c.f.u/g for higher risk products (eye, baby products, and others) and <1000 c.f.u /g for all other products. However, all cosmetic products must be devoid of pathogenic microorganisms, such as, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans* and *E. coli* [3].

Evidence of microbial contamination and spoilage. Microbial contamination of cosmetics renders them unfit for use as the products may develop various degree of aesthetic changes. The growth of bacteria that produces alcohols or degrades emulsifiers may lead to instability, splitting of the emulsion and eventual spoilage. Microbial growth can produce enzymes that causes degradation of active ingredients and changes in the pH. Cellulose polymers in cosmetics can act as targets for microbial attack, and support extensive growth under suitable conditions when converted from a stiff gel into a running liquid, thereby rendering the cosmetic unfit for use. Finally, changes in the products may become evident as unpleasant aromas and tastes, color changes, production of irritants, loss of activity, alterations in viscosity, bulging/leakage of products, and visible surface growth [4].

Impact of Microbial contamination may include the cost of disposal and replacement of contaminated products, if a recall is required, loss of reputation to the brand or the manufacturer, serious microbial skin infections and even blindness, if the product is applied around the eyes [5]. A link between outbreak of Pseudomonas aeruginosa in a neonatal intensive care unit was reported [6] in the USA with contaminated hand washing lotion, just as Kallings et al. [7] reported that hydrocortisone ointment contaminated by Pseudomonas spp resulted in severe eye infections when used in the treatment of an ophthalmic condition. An outbreak of invasive mycoses caused by Paecilomyces lilacinus from contaminated skin lotion was reported by Orth et al. [8] in Switzerland, the cutaneous manifestation of which was confirmed [9]. Consequently, the health implications of microbially contaminated cosmetic products necessitated the US Department of Health and Human Services to issue a serious Import Alert (code named "Detention without Physical Examination of Cosmetics Due To Microbiological Contamination") on some products. The scope of the products included the followings among others: (1) Import alert on 15th June 2010 on Baby Moisturizing Lotions, Creams and Powders contaminated by Enterobacter gergoviae, and manufactured by Bio Botanical Labs, Quebec, Canada; (2) Import Alert on 23rd June 2011, on eye shadow and Lip gloss cosmetics contaminated by Staphylococcus intermedius and S. warneri and manufactured by beauty vision limited, Gong ming shenzen, China; (3) Import Alert of 7th Dec 2010 on Alexia Lip gloss cosmetic contaminated by Sphingomonas paucimobilis and Pseudomonas aeruginosa, manufactured by Maesa, Jinwan Zhuhai [10].

Previous reports of microbial quality assessment of cosmetics and toiletries were mainly from developed countries [11] usually in response to outbreaks of infectious diseases [6]. In the African continent, particularly Nigeria, few studies

have been carried out to evaluate the microbiological quality of many brands of commercial cosmetics products that flood the Nigerian market, from the local and International cosmetic producers. Of these studies, Hugbo et al. [12] evaluated the microbial quality of ten brands of commercially available creams and lotions in Benin city Nigeria and discovered Staphylococci, Penicillium, Aspergillus fumigatus and Microsporium species as the major contaminants. Similarly, Okorie [13,14] in separate studies reported the presence of Klebsiella, Pseudomonas aeruginosa, Bacillus spp, Staphylococcus aureus and Penicillium spp from some brands of commercially hawked cosmetics products in Nigeria. However, these previous reports were primarily focused on products consumed by adults, and none of them provided the morphological characteristics of the detected fungi.

Hair straightener otherwise known as ‘‘relaxer’’ is a type of lotion or cream generally used by people with "afro textured hair", to make hair less curly, easier to straighten or to create perms by chemically "relaxing" the natural curls by breaking down the proteins bonds of hair, temporarily or permanently.

Lip gloss is a brand of cosmetic product invented by Max Factor in 1930, primarily used to give lips a glossy lustre and subtle color. It is distributed as a liquid or a soft solid, completely clear, translucent, or various shades of opacity, including frosted, glittered, glassy, and metallic finishes, packaged in squeezable tube and wand applicator formats such as “Lip Smackers” [15].

The harmful microbes in cosmetics constitute a much greater risk to new born babies, infants, and young children, than to adolescents and adults [16,17]. Considering the neglected research on the ecology and colonization of cosmetics by pathogens, this study investigated the microbiological quality of popular brands of Baby lotions, Hair relaxers, and Lip gloss cosmetics in Nigeria, and determined the characteristics of the detected fungal and bacteria contaminants.

2. MATERIALS AND METHODS

2.1 Samples

A total number of forty-five (45) cosmetic products representing (15) units each of; ‘‘Oz’’ hair relaxer, “Lip gloss” and ‘‘P’’ baby lotion brands of cosmetics, were randomly purchased from different retail outlets in Southwest, Nigeria. Some of the products had comparable batch numbers, date of production, expiry dates and National Agency for Food, Drug Administration and Control (NAFDAC) numbers on the products labels. None of the products was expired prior to analysis at the Microbiology Laboratory of Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria. The physical attributes of the products were studied and other information obtainable from the product labels were recorded.

2.2 Microbiological Analysis of Samples

The methods of Hitchings et al. [18] was adopted in evaluating the viable bacteria and fungal colonies, under a laminar air flow cabinet. After surface sterilization of the container with 70% ethanol, using a sterile spatula or pipette, 1 gram (or 1 ml) of each sample was aseptically withdrawn and dissolved in 1 ml sterile Tween-80 diluent, vortexed, made up to 10^{-1} dilution in 8 mls of sterile Modified Letheen Broth (MLB), from which 5 mls was diluted in 45 mls of MLB to obtain 10^{-2} dilution, and further serially diluted to 10^{-4} dilution. Employing the pour plate method, Inoculum (1 ml) was taken from 10^{-2} dilution to seed sterile plates of replicated Tryptic Soy Agar (TSA), Modified Letheen Agar (MLA) and Mannitol Salt Agar (MSA) and Chloramphenicol supplemented Sabouraud Dextrose Agar (SDA). The plates with uninoculated growth controls were then allowed to solidify, inverted and incubated at 37°C for 48hrs (TSA, MSA and MLA) and SDA at 27°C for between 3-5 days.

2.3 Characterization and Identification of the Bacterial Isolates

The bacteria isolates were characterized on the basis of their cellular morphology, cultural, biochemical, and sugar fermentation according to Olutiola et al. [19] and identified according to Holt et al. [20,21].

2.4 Antibiotic Susceptibility of the Bacterial Isolates

The antibiotic susceptibility of the bacteria was determined using the standard method of CLSI [22]. The following antibiotics and their disk concentrations were tested against the isolates using the Kirby-Bauer disk diffusion method on Mueller-Hinton Agar (Oxoid). Predetermined commercial (Oxoid) Gram positive bacteria antibiotic discs (Augmentin 30 µg, Amoxicillin 25 µg, Erythromycin 5 µg, Tetracycline 10 µg, Cloxacillin 5 µg, Gentamicin 10 µg,

Cotrimoxazole 25 µg and Chloramphenicol 30 µg) were applied to the surface of the inoculated agar plates using a pair of sterile forceps. For the Gram negative isolates, antibiotic discs (Ampicillin 25 µg; Cotrimoxazole 25 µg; Gentamicin 10 µg; Nalidixic acid 30 µg; Nitrofuratoin 200 µg; Colistin 25 µg; Streptomycin 25 µg and Tetracycline 25 µg) were applied to determine the phenotypic antibiotic resistance of the isolates.

2.5 Characterization and Identification of the Fungal Isolates

Typical discrete pure culture of each isolate was selected, characterised and identified by combinations of the cultural and microscopic observations/characteristics. The isolates were further identified based on the microscopic fungal features such as Phialides, septate Sporangiophores, Conidiophores, and budding, as previously described [23]. In addition, typical isolates were confirmed by culturing the pure isolates on 2% Malt Extract Agar to achieve enough conidiation, from which suspensions were prepared and inoculated into the BIOLOG FF Microplate (BIOLOG, CA, USA) and incubated at 26°C for 5days. Characteristic fingerprints of each isolate were then read using the BIOLOG Microstation and confirmed with the FF Database. Furthermore, the fungal wet mounts were prepared using Lactophenol-in-cotton blue, viewed under the microscope at X400 mag. and the photomicrographs were digitally recorded. The observed characteristics were then compared and confirmed with reference to Ellis [24].

3. RESULTS AND DISCUSSION

3.1 Microbiological Quality of the Products

Of the total number of forty five (45) samples representing three popular brands of selected cosmetic products that were analyzed in this research, thirty- eight samples (84%) were positive for various levels of microbial contaminants (Tables 1 and 6). The qualitative microbiological analysis showed that 15 samples of the Lip Gloss, 12 samples of the Baby Lotion, and 11 samples of the Relaxer, were contaminated to varying degrees with respect to the diversity, the counts, and presence of potential bacterial and fungal pathogens. The bacteria counts in the Baby lotion ranged between $3.5x10^2$ and $55x10^2$ cfu/ml, in the Relaxer the counts were between $3.5x10^2$ and $7.7x10^2$ cfu/ml, while the counts in the Lip Gloss were $20x10^2$ to $38x10^2$ cfu/ml respectively. No contaminant was detected in samples R5, R8, R11, R13, P2, P9 and P15. While the product labels of the baby lotion and the Hair Straightener had authentic production dates, batch numbers, expiry dates, and NAFDAC numbers, all these information were lacking on the Lip Gloss samples. There were no visible defect(s) on the samples prior to the analysis. Regarding the shelf life of the products, a high fungal and bacteria counts were recorded as some of them were closer to the expiry dates (Table 1). The shelf life of the Lip Gloss could not be determined as the necessary information were lacking on the product labels, thereby making vital assessment and batch recalls (of such products) impossible.

3.2 Microbial Diversity

Seventy (70) microbial isolates belonging to eleven (11) bacteria genera (comprising 7 aerobic Gram positive rods, 10 Gram positive cocci, and 27 Gram negative bacteria), and nine (9) fungal genera were isolated from 84% of the contaminated cosmetic samples (Tables 2 and 6). The percentage occurrence of the predominant contaminants from the Lip gloss cosmetic were *Streptococcus lactis* (40%), *Klebsiella pneumoniae* (11.1%), *Staphylococcus aureus* (40%), *Bacillus licheniformis* (28.6%), *Bacillus cereus* (14.2%), *Enterobacter* spp (3.7%), *Erwinia carotovora* (3.7%), *Micrococcus luteus* (10%) and *Escherichia hermannii* (3.7%), while the fungi included *Aspergillus niger* (3.8%), *Penicillium* spp (3.8%), and *Aspergillus flavus* (7.7%). From the hair relaxers, the predominant bacteria were *Erwinia amylovora* (11.1%), *Serratia rubidaea* (11.1%), *Bacillus licheniformis* (28.6%), *Buttiauxella agrestis* (7.4%) and*Pseudomonas putida* (3.7%), while *Aspergillus fumigatus* (3.8%), *Candida* spp (3.8%), *Aspergillus glaucus* (3.8%), *Cladosporium* spp (7.7%) *and Trichoderma herzianum* (3.8%) constituted the fungi. The bacteria contaminants in the Baby lotion were *Erwinia amylovora* (11.1%), *Serratia marcescens* (3.7%), *Streptoococcus lactis* (10%), *Enterobacter gergoviae* (18.5%), *Bacillus subtilis* (28.6%), *Pseudomonas aeruginosa* (7.4%) and *Enterobacter cloacae* (3.7%), while the fungi were *Aspergillus nidulans* (7.7%), *Penicillium* spp (7.7%), *Trichoderma* spp (3.8%), *Candida albicans* (3.8%), *Rhizopus* spp (3.8%), *Aspergillus flavus* (7.7%), *Mucor mucedo* (3.8%), *Fusarium solanii* (3.8%), *Fusarium* spp (7.7%) and *Geotrichum* spp (7.7%) (Tables 5 and 6).

Table 1. Physical attributes and the total viable counts (TVC) of the bacteria isolates from commercial Hair straightener (Relaxer), Lip gloss and baby lotion cosmetics

Samples	Production Date	Batch No	Expiry date	NAFDAC No	Visible defects	Date of analysis	TVC (cfu/g)
Relaxer							
R1	29/05/10	BV080	29/11/11	+	Nil	Aug. 2011	$5.5x10^2$
R2	29/05/10	BV080	29/11/11	+	Nil	Aug. 2011	$4x10^2$
R3	24/07/10	QU110	29/02/12	+	Nil	Aug. 2011	$3.5x10^2$
R4	23/08/10	QU009	29/02/12	+	Nil	Aug. 2011	$3.5x10^2$
R6	23/08/10	QU000	29/02/12	+	Nil	Aug. 2011	$3.8x10^2$
R7	29/05/10	BV080	29/11/11	+	Nil	Aug. 2011	$4.7x10^2$
R9	29/05/10	BV080	29/11/11	+	Nil	Aug. 2011	$4.2x10^2$
R10	23/08/10	QU009	29/02/12	+	Nil	Sept. 2011	$3.5x10^2$
R12	29/05/10	BV085	29/11/11	+	Nil	Sept. 2011	$4.5x10^2$
R14	27/05/10	Nil	29/11/11	+	Nil	Sept. 2011	$6.7x10^2$
R15	27/05/10	Nil	29/11/11	+	Nil	Sept. 2011	$7.7x10^2$
Lip gloss							
LG1	Nil	Nil	Nil	Nil	Nil	Sept. 2011	$30x10^2$
LG2	Nil	Nil	Nil	Nil	Nil	Sept. 2011	$35x10^2$
LG3	Nil	Nil	Nil	Nil	Nil	Sept. 2011	$20x10^2$
LG4	Nil	Nil	Nil	Nil	Nil	Sept. 2011	$31x10^2$
LG5	Nil	Nil	Nil	Nil	Nil	Sept. 2011	$20x10^2$
LG6	Nil	Nil	Nil	Nil	Nil	Sept. 2011	$20x10^2$
LG7	Nil	Nil	Nil	Nil	Nil	Sept. 2011	$30x10^2$
LG8	Nil	Nil	Nil	Nil	Nil	Sept. 2011	$22x10^2$
LG9	Nil	Nil	Nil	Nil	Nil	Sept. 2011	$25x10^2$
LG10	Nil	Nil	Nil	Nil	Nil	Sept. 2011	$23x10^2$
LG11	Nil	Nil	Nil	Nil	Nil	Oct. 2011	$28x10^2$
LG12	Nil	Nil	Nil	Nil	Nil	Oct. 2011	$27x10^2$
LG13	Nil	Nil	Nil	Nil	Nil	Oct. 2011	$36x10^2$
LG14	Nil	Nil	Nil	Nil	Nil	Oct. 2011	$35x10^2$
LG15	Nil	Nil	Nil	Nil	Nil	Oct. 2011	$38x10^2$
Baby lotion							
P3	28/06/2011	08:31	28/12/2012	+	Nil	Sept 2011	$3.5x10^2$
P4	28/04/2011	14:13	28/10/2012	+	Nil	Sept 2011	$3.5x10^2$
P5	28/04/2011	14:12	28/10/2012	+	Nil	Sept 2011	$3.8x10^2$
P6	20/05/2011	07:21	20/11/2012	+	Nil	Sept 2011	$3.5x10^2$
P7	28/04/2011	14:14	28/10/2012	+	Nil	Sept 2011	$4.3x10^2$
P8	28/04/2011	14:12	28/10/2012	+	Nil	Sept 2011	$4.5x10^2$
P10	28/04/2011	14:13	28/10/2012	+	Nil	Sept. 2011	$3.8x10^2$
P11	28/04/2011	14:12	28/10/2012	+	Nil	Sept. 2011	$4.5x10^2$
P12	20/05/2011	07:21	20/11/2012	+	Nil	Sept. 2011	$3.5x10^2$
P13	29/04/2011	08:30	29/10/2012	+	Nil	Oct. 2011	$4.5x10^2$
P14	27/04/2011	14:12	27/10/2012	+	Nil	Oct. 2011	$3.5x10^2$

Enterobacter gergoviae, Serratia marcescens, Pseudomonas aeruginosa, and *Enterobacter cloacae* from the baby lotion were all susceptible to Nitrofurantoin, Nalidixic acid, Gentamicin, Cotrimoxazole, and Streptomycin, but resistant to Tetracycline, Colistin and Ampicillin. *Klebsiella pneumoniae* was only susceptible to Nalidixic acid, Gentamicin, Tetracycline, Cotrimoxazole and Streptomycin, as shown in Table 3. All values <12-14 mm indicated Resistant, between 13-15 mm were Intermediate, while values >16 mm indicated Susceptibility to the Antibiotics; Nd: not determined.

Table 2. Identities of bacteria isolates from commercial cosmetics products

Samples	Isolates	Identity	Samples	Isolates	Identity
R1	R1A	*Erwinia amylovora*	LG9	LG9A	*Staphylococcus aureus*
R2	R2A	*Serratia rubidaea*	LG10	LG10A	*Enterobacter spp*
R3	R3A	*Bacillus licheniformis*	LG11	LG11A	*Erwinia carotovora*
R4	R4A	*Serratia rubidaea*	LG12	LG12A	*Staphylococcus aureus*
	R4B	*Buttiauxella agrestis*	LG13	LG13A	*Micrococcus luteus*
R6	R6A	*Serratia rubidaea*	LG14	LG14A	*Escherichia hermannii*
R7	R7A	*Erwinia amylovora*	LG15	LG15A	*Staphylococcus aureus*
R9	R9A	*Buttiauxella agrestis*	P1	P1A	*Erwinia amylovora*
R12	R12A	*Erwinia amylovora*	P3	P3A	*Serratia marcescens*
R14	R14A	*Bacillus licheniformis*		P3B	*Staphylococcus lactis*
R15	R15B	*Pseudomonas putida*	P4	P4A	*Enterobacter gergoviae*
LG1	LG1A	*Streptococcus lactis*		P4B	*Erwinia amylovora*
LG2	LG2A	*Klebsialla pneumonia*	P5	P5B	*Bacillus subtilis*
	LG2B	*Staphylococcus aureus*	P6	P6A	*Bacillus subtilis*
	LG2C	*Streptococcus lactis*		P6B	*Enterobacter gergoviae*
	LG2D	*Bacillus licheniformis*	P7	P7A	*P. aeruginosa*
LG3	LG3A	*Streptococcus lactis*	P8	P8A	*Enterobacter gergoviae*
LG4	LG4A	*Streptococcus lactis*	P10	P10A	*Enterobacter gergoviae*
LG5	LG5A	*Klebsiella pneumonia*	P11	P11A	*Enterobacter gergoviae*
LG6	LG6A	*Klebsialla pneumonia*	P12	P12B	*P. aeruginosa*
LG7	LG7A	*Bacillus licheniformis*	P13	P13A	*Enterobacter cloacae*
LG8	LG8A	*Bacillus licheniformis*	P14	P14A	*Erwinia amylovora*
	LG8B	*Bacillus cereus*			

Table 3. Antibiogram of Gram negative bacterial isolates from commercial cosmetics products

Isolates (Codes)	Zones of Inhibition by the Antibiotics (mm)							
	NIT	NAL	GEN	TET	COL	AMP	COT	STR
Erwinia amylovora (R1A)	12	15	21	02	02	05	19	02
Serratia rubidaea (R2A)	00	09	21	19	00	00	23	00
Serratia rubidaea (R4A)	00	09	19	20	00	00	24	02
Buttiauxella agrestis (R4B)	00	23	25	23	00	00	00	02
Serratia rubidaea (R6A)	00	11	22	20	00	00	23	01
Erwinia amylovora (R7A)	08	00	13	00	05	00	05	00
Buttiauxella agrestis (R9A)	00	22	24	22	00	01	01	02
Erwinia amylovora (R12A)	17	21	14	01	04	01	04	01
Pseudomonas putida (R15B)	18	20	18	05	04	05	20	10
Klebsiella pneumoniae(LG2A)	00	20	18	20	04	01	18	20
Klebsiella pneumoniae LG5A)	00	20	18	20	04	00	17	20
Klebsiella pneumoniae(LG6A)	00	21	17	21	05	00	16	20
Enterobacter spp (LG10A)	18	19	14	02	02	01	17	19
Erwinia carotovora (LG11A)	00	02	11	00	00	00	05	00
Escherichia hermannii(LG14A)	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Erwinia amylovora (P1A)	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Serratia marcescens (P3A)	22	22	20	20	13	00	00	00
Enterobacter gergoviae (P4A)	17	21	01	01	00	00	17	21
Erwinia amylovora (P4B)	08	00	12	00	05	00	05	00
Enterobacter gergoviae (P6B)	17	22	17	00	00	00	17	22
Pseudomonas aeruginosa (P7A)	20	22	17	09	00	00	00	07
Enterobacter gergoviae (P8A)	16	22	17	01	00	00	17	22
Enterobacter gergoviae (P10A)	17	22	17	00	00	00	17	22
Enterobacter gergoviae (P11A)	16	21	16	01	00	00	18	21
Pseudomonas aeruginosa (P12A)	20	21	16	01	01	01	01	06
Enterobacter cloacae (P13A)	21	21	17	00	00	00	00	00
Erwinia amylovora (P14A)	08	00	11	00	05	00	04	00

Among the Gram positive isolates, 52.9% comprising *Staphylococcus aureus, Streptococcus lactis,* and *Micrococcus luteus,* were susceptible to Erythromycin, Amoxicillin, Gentamicin, Cotrimoxazole, and Tetracycline but resistant to Chloramphenicol and Augmentin (Table 4).

4. DISCUSSION

Statutory Cosmetic Industry standards stipulated a total viable count of aerobic bacteria, yeast and moulds of less than 100 c.f.u/g (or ml) for eye and baby products and 1000 c.f.u/g (or ml) for other products, at completion of manufacture, while potentially pathogenic microorganisms such as *Pseudomonas*, *Candida* and *Staphylococcus* should not be present in the product [3]. The results of this study clearly indicated that the microbial diversity and population of the detected organisms exceeded the stipulated permissible levels, particularly in the Baby lotion and the "Lip gloss". These findings therefore disqualify these products as meeting the stipulated qualitative microbial limits in Official monographs [3]. The manufacturers needed to evolve adequate measures towards the equipment cleaning and sanitation, as the observed contamination could be due to periodic dislodgements from equipment surfaces, crevices, elbows and joints. Although, the bacteria diversity was highest in the lip gloss, it however contained the least fungal diversity. Four (4) fungal genera were detected from the Hair Relaxers, as opposed to the eight (8) and two (2) genera that were detected in the Baby lotions and the Lip gloss respectively. This may be attributed to the alkalinic nature of the products. While fungi grows best in acidic milieu as obtained in the Baby lotion, the bacteria prefers alkaline and neutral medium as obtainable in the Hair relaxers and the Lip gloss. Hence, the bacteria diversity exceeded the fungal diversity in all the product categories as presented in Tables 1, 2 and 5.

Fungal colonization and multiplication in cosmetic products are more rapid and evident than bacteria because of fungi nutritional and enzymatic versatilities, proliferation and eventual spoilage of the products. Three fungal enzymes (exo-β-1, 4-glucanase, endo-β-1,4-glucanase and β-glucosidase) usually synthesized by three fungal species, belonging to the genera *Aspergillus, Fusarium* and *Penicillium,* are involved in the degradation of crystalline cellulose in cosmetics and toiletries [25]. These groups of fungi were typically isolated in this study (Table 5). *Cladosporium* species may present opportunistic infection in the user as it was reported to cause infections of the skin and toenails, and may effect spoilage of the hair relaxer product as it was incriminated in the transformation of some steroidal drug to inactive forms and offensive metabolites [26].

Table 4. Antibiogram of gram positive bacteria from commercial cosmetics products

Isolates (Codes)	Zones of inhibition by the antibiotics (mm)							
	TET	CXC	GEN	COT	CHL	AUG	AMX	ERY
Bacillus licheniformis (R3A)	00	22	14	04	00	00	00	00
Bacillus licheniformis (R14A)	00	09	19	20	00	00	24	02
Streptococcus lactis (LG1A)	00	21	26	11	01	15	00	27
Staphylococcus aureus (LG2B)	24	12	34	14	12	18	23	15
Streptococcus lactis (LG2C)	21	17	27	13	00	15	25	11
Streptococcus lactis (LG3A)	00	21	27	12	02	16	01	28
Streptococcus lactis (LG4A)	00	20	26	11	00	00	00	27
Bacillus licheniformis (LG7A)	00	09	18	20	00	00	22	02
Bacillus licheniformis (LG8A)	00	09	19	20	00	00	23	02
Bacillus cereus (LG8B)	06	00	00	00	00	00	06	10
Staphylococcus aureus (LG9A)	23	12	33	13	12	20	22	15
Staphylococcus aureus (LG12A)	24	12	34	14	12	22	23	15
Micrococcus luteus (LG13A)	15	20	22	15	05	15	18	18
Staphylococcus aureus (LG15A)	23	11	35	13	12	01	22	16
Streptoococcus lactis (P3B)	00	16	00	00	15	00	00	00
Bacillus subtilis (P5B)	00	00	12	00	24	00	00	00
Bacillus subtilis (P6A)	00	16	00	00	23	00	06	15

Table 5. The fungal counts, colonial morphology, cellular morphology and the identities of fungal contaminants from commercial Hair straighthener, Lip Gloss and Baby lotion cosmetics

Sample	Isolate	Fungal count	Colonial morphology	Cellular morphology	Identity
R1	R1A	$20x10^2$	Colonies show cottony suede like surface typical blue-green.	Conidial heads typical columnar. Conidiophores short and smooth-walled. Conidia are produced in basipetal succession forming long chains. Conidia are globose to subglobose, green and rough-walled.	*Aspergillus fumigatus*
	R1FB	$4.5x10^2$	Colonies white to cream color, smooth and yeast-like in appearance.	Oval budding yeast cells, produced pseudo-mycelium apically or laterally.	*Candida* spp
R2	R2FA	$10x10^2$	Dirty yellowish colony.	Conidiophores hyaline shades of brown, metulae absent, conidia subglobose, arising circumferentially from spherical vesicle.	*Aspergillus glaucus*
R3	R3FA	$3.5x10^2$	Grayish green, flat elevation.	Presence of shield-shaped conidia, pale brown conidiophores are less distinct from vegetative hyphae, tree –like branching.	*Cladosporium* spp
R4	R4FA	$4x10^2$	Grayish green, flat elevation.	Presence of shield-shaped conidia, pale brown, conidiophores less distinct from vegetative hyphae.	*Cladosporium* spp
	R4FB	$4.5x10^2$	Colonies yellowish . Circular, lobate, moderate, orange, growing dull blue green, reverse slightly greenish.	Conidiophores short and thick, branching, short and plump phialide with conidia at the tip.	*Trichoderma herzianum*
LG1	LG1A	$2.5x10^2$	Pale yellow colonies turning jet-black, covered by a dense layer of dark conidial heads. Reverse of plate was yellowish gray.	Large, globose, dark brown conidial heads, which become radiate. Conidiophores smooth walled and hyaline. Aseptate hyphae. Conidia heads globose biserate, dark brown with phialides borne on septate metulae circumferentially.	*Aspergillus niger*
LG2	LG2A	$2.5x10^2$	Colonies are usually shades of blue, green and white.	Mycelium highly branched, hyphae is colorless, septate. conidiospores green in color.	*Penicillium* spp
	LG2B	$13x10^2$	Light brown, Loosely interwoven mycelia with white patches. The reverse was golden to reddish brown.	Conidiophores are coarsely roughened. Conidia are globose to subglobose. Conidia heads loosely columnar, cream bluff, conidiophores stipes yellow to brown, biseriate.	*Aspergillus flavus*
	LG2C	$4.5x10^2$	Colonies with radial grooves, granular velvety, yellowish with white patches at the edge.	Conidia heads typically radiate, Conidia round, smooth formed long chains, conidiophores long, hyaline, phialides are biseriate.	*Aspergillus flavus*

Sample	Isolate	Fungal count	Colonial morphology	Cellular morphology	Identity
P1	P1FA	$4.5x10^2$	Colonies velvety, circular, entire, large, yellowish- brown. Reverse was red-brown.	Rough walled globose conidia on short brownish, smooth walled conidiophores. Conidial heads are short columnar and biseriate. Phialides on distal portion of vesicle.	*Aspergillus nidulans*
	P1FB	$3.5x10^2$	Irregular, Lobate, Moderate, Mint green. Reverse yellowish green. Colonies usually growing dull blue green.	short simple condiophores rising from aerial hyphae, conidia forming loose, brush-like columns, ellipsoidial to subglobose, smooth walled.	*Penicillium* Spp
P2	P2FA	$4x10^2$	Entire with white patches at the edge, Lobate, Moderate, yellowish green. colonies usually growing dull blue green.	Short condiophores arising from aerial hyphae,. Conidia forming loose brush-like columns, ellipsoidial to subglobose, smooth walled.	*Penicillium* spp.
	P2FB	$4.5x10^2$	Colonies velvety, circular, entire, large, yellowish- brown. Reverse was reddish-brown.	Rough- walled globose conidia on short brownish smooth walled conidiophores. Short columnar and biseriate conidial heads. Conidia were sometimes hyaline.	*Aspergillus nidulans*
	P2FC	$3.5x10^2$	Circular, lobate, moderate, orange, colonies growing dull blue green, reverse slightly greenish.	Short simple, condiophores rising from aerial hyphae,. Conidia forming loose columns, ellipsoidial to subglobose, smooth walled. branching, short and plump phialide.	*Trichoderma* spp
P3	P3FA	$14x10^2$	Circular, small, shiny milky colony opaque with a diameter of 1-3mm in size.	single cells, oval or cylindrical in shape.	*Candida albicans*
	P3FB	$2x10^2$	Large, filamentous grey colored colony.	Colonies are very fast growing with some tendency to collapse, pale or dark brown grey stolons hyaline. Smooth-walled non-septate sporangiophores arising from stolons opposite the rhizoids.	*Rhizopus* spp.
	P3FC	$2x10^2$	Filamentous, large, yellowish colored colony.	Conidia heads typically radiate, Conidia round, smooth formed long chains, conidiophores long, phialides are biseriate. It has typically yellow green conidiophores that are simple terminating, in clavate swelling.	*Aspergillus flavus*
	P3FD	$3x10^2$	Circular, moderate, silvery gray mycelia.	Wide sporangiophores and a denser layer of short repeatedly branch sporangiophores. Sporangia on tall sporangiophores brownish gray at maturity, wall spinulose.	*Mucor mucedo*

Sample	Isolate	Fungal count	Colonial morphology	Cellular morphology	Identity
P4	P4FA	$5.3x10^2$	Circular, small, shiny, milky.	Single cells, usually spherical, oval or cylindrical in shape. Colonies were cream, opaque and soft of diameter 1-3mm in size.	*Candida* spp
	P4FB	$25x10^2$	Circular, lobate, moderate, yellowish green. Colonies reaching 5.5cm in diameter.	Conidia heads typically radiate, Conidia round, smooth formed long chains, conidiophores long, hyaline, phialides are biseriate	*Aspergillus flavus*
	P4FC	$30x10^2$	Circular, entire, large, wooly, white colony. Reverse brownish rhizoidal.	Conidiophores, slender and simple bearing a whorl of phialides, with macroconidia. several celled and slightly curved at the pointed end. Dispersed from the conidiophores.	*Fusarium solanii*
P5	P5FA	$4.5x10^2$	Rapidly growing, circular, entire, large, white colony. Mycelium extensive, thick and cottony in culture, with some tinge of pink.	Conidiophores, slender and simple bearing phialides, with macroconidia held in small mont head that are several celled and slightly curved at the pointed end.	*Fusarium* spp
	P5FB	$2x10^2$	Mycelium white, circular, entire, large,	Septate, conidiophores absent, conidia hyaline. One-celled, short, cylindrical with truncate ends, formed by segmentation of hyphae.	*Geotrichum* spp
P6	P6FA	$12x10^2$	Circular, entire, large, white colony. Mycelium extensive and cottony in culture, with some tinge of pink.	Conidiophores, slender and simple bearing phialides, with macroconidia held in small mont head that are several celled and slightly curved at the pointed end.	*Fusarium* spp
	P6FB	$3.5x10^2$	Mycelium white, Circular, entire, large, thick and velvety.	Septate, conidiophores absent, conidia hyaline. One-celled, short, cylindrical with truncate ends, formed by segmentation of hyphae.	*Geotrichum* spp

Table 6. The number, percentage of isolates and contaminated samples of the commercial cosmetics products

Isolated contaminants	Cosmetic brand (% of contaminated samples)			TOTAL
	Relaxer (73.3%)	Lip gloss (100%)	Baby lotion (80%)	38/45 (84.4%)
	Number of isolate (%)	Number of isolate (%)	Number of isolate (%)	
G +VE RODS				
Bacillus licheniformis	2 (28.6)	2 (28.6)	-	
Bacillus cereus	-	1 (14.2)	-	
Bacillus subtilis	-	-	2 (28.6)	
SUB-TOTAL:	2	3	2	7
G +VE COCCI				
Streptococcus lactis	-	4 (40)	1 (10)	
Staphylococcus aureus	-	4 (40)	-	
Micrococcus luteus	-	1 (10)	-	
SUB-TOTAL:	0	9	1	10
G -VE RODS				
Erwinia amylovora	3 (11.1)	-	3 (11.1)	
Erwinia carotovora	-	1 (3.7)	-	
Serratia rubidaea	3 (11.1)	-	-	
Buttiauxella agrestis	2 (7.4)	-	-	
Pseudomons putida	1 (3.7)	-	-	
Klebsiella pneumoniae	-	3 (11.1)	-	
Enterobacter spp	-	1 (3.7)	-	
Escherichia hermannii	-	1 (3.7)	-	
Serratia marcescens	-	-	1 (3.7)	
Enterobacter gergoviae	-	-	5 (18.5)	
Pseudomonas aeruginosa	-	-	2 (7.4)	
Enterobacter cloacae	-	-	1 (3.7)	
SUB-TOTAL:	9	6	12	27
FUNGI				
Aspergillus fumigatus	1 (3.8)	-	-	
Candida spp	1 (3.8)	-	1 (3.8)	
Aspergillus glaucus	1 (3.8)	-	-	
Cladosporium spp	2 (7.7)	-	-	
Trichoderma herzianum	1 (3.8)	-	-	
Aspergillus niger	-	1 (3.8)	-	
Penicillium spp	-	1 (3.8)	2 (7.7)	
Aspergillus flavus	-	2 (7.7)	2 (7.7)	
Aspergillus nidulans	-	-	2 (7.7)	
Trichoderma spp	-	-	1 (3.8)	
Candida albicans	-	-	1 (3.8)	
Rhizopus spp	-	-	1 (3.8)	
Mucor mucedo	-	-	1 (3.8)	
Fusarium solanii	-	-	1 (3.8)	
Fusarium spp	-	-	2 (7.7)	
Geotrichum spp	-	-	2 (7.7)	
SUB-Total:	6	4	16	26
Total	17	22	31	70

The detection of these fungal contaminants (Figs. 1a -1u), particularly the pathogenic species comprising *Candida, Fusarium, Aspergillus* and *Trichoderma* cannot be condoned in the “P” Baby

lotion as this brand is a popular product applied in the personal care of the newborn (> 28 days old), the infants (1year old) and the children (1-4 year old), whose immune developments were still rudimentary. The recent increase in numbers of people with immunodeficiencies has caused an equally dramatic surge in the incidences of fungal infections particularly *Candida*, *Aspergillus* and *Penicillium* species. Aspergillosis is recognized as the most common fungal infection of the immunocompromised patients, while *Candida* sp. is one of the notorious pathogens responsible for septicemia and superficial mycotic infections of the skin, the nail, the hair, and mucous membranes. Physiologically, the acidic pH of the human skin ranges between 4-5, while the acidic growth condition of fungi ranges between 2.2 - 4.5. These pH ranges potentially provide favorable conditions for superficial infection and invasive establishment of fungal diseases in the tender skin of the susceptible user. Furthermore, majority of the detected fungi have the capacity to elicit mycotic keratitis, an opportunistic fungal infection of the eye that causes ulceration and inflammation, usually following a trauma to the cornea by vegetative matter, contaminated cosmetics, soil, surgery, or prolonged treatment with corticosteroids by some users who also apply toning or bleaching creams [27]. The etiological agents include various saprophytic fungi especially *Aspergillus fumigatus*, *A. flavus*, *A. niger*, *Fusarium solani* and *Candida albicans*, that were typically isolated in this present study. It is also possible that epithelial damage caused by *Aspergillus* could release nutrients for fungal growth in infected tissues.

Aside the direct pathogenic infection of these fungal contaminants, the isolates synthesizes secondary metabolites directly into their growth milieu. Of these metabolites is gliotoxin of *Aspergillus fumigatus*, with potent cilioinhibitory, genotoxic and cytotoxic properties, produced by various *Penicillium*, *Aspergillus*, *Gliocladium*, *Thermoascus*, and *Candida* species [28]. Intrinsic contamination and in-use growth of these contaminants overtime, will certainly elicit the metabolites. Although it is not yet known how common the production of gliotoxin exist in the actual cosmetics from which *A. fumigatus*, *Penicillium* and *Candida* were isolated, or to what extent and by what kind of mechanism the gliotoxin could be mobilized from the products. However, the fact remains that the contaminated cosmetics present potential risk to the health of the consumer.

A considerable growth area in marketing cosmetics is specialized ethnic populations, of products targeting the African-American, Hispanic, and Asian-American markets. In the ethnic market, products that target skin decoloration are experiencing the best consumer response [29,30]. However, it was a common phenomenon and gory sight to behold in some consumers, who in the name of civilization, apply bleaching and toning creams to achieve lighter complexion [16], but eventually acquire patchy, scaly, chameleonic complexion, attributable to the synergistic impact of the chemical constituents and microbial contaminants in the formulations. As creams consist of emulsions of water and oil, stabilized with emulsifiers, they are prone to contamination with *Pseudomonas*, *Burkholderia cepacia*, *Micrococci*, *Staphylococcus aureus,* and aerobic spore formers [4]. These conditions may have been responsible for the detection of *Pseudomonas aeruginosa* and high prevalence of *Enterobacter gergoviae, Bacillus subtilis* and *Serratia marcescens* in the Baby lotion. The mesophilic nature of *Bacillus* is enhanced by the storage temperature and retailed conditions of the products. Some microorganism survive by forming endospores, biofilms, capsules, extracellular enzymes and by exhibiting acid tolerance [31]. All these characteristics are exceptionally exhibited by the isolated bacteria species in this study and could have been responsible for their survival in the cosmetic products, particularly *Buttiauxella agrestis* which is hereby reported for the first time in the Hair straightener (Relaxer) brand.

Generally, these products from where *Pseudomonas, Bacillus*, *Klebsiella*, *Staphylococcus, Escherichia*, *Enterobacter* and *Candida* were isolated should not have been released for consumers' use, in view of the pathogenic potentials. Similar discovery of these potentially pathogenic species prompted the US Department of Health and Human Services to issue a serious ''Import Alert'' on some cosmetic products [10]. The detection of *Escherichia hermannii, Staphylococcus aureus, Bacillus cereus,* and *Enterobacter* spp in the ''Lip Gloss'' portends a critical risk to health as the cosmetics are usually applied on the lips and may eventually be ingested and elicit gastroenteric infections [32] in the susceptible user. Typical microbial contamination by objectionable microorganisms that necessitated product recalls of some baby lotions, creams, shampoos

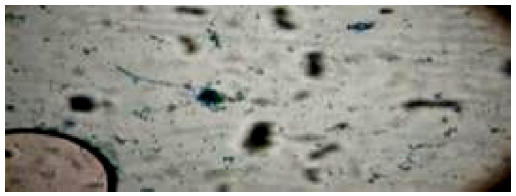

Fig. 1a. Micrograph of *Aspergillus fumigatus* (R1FA) isolated from Hair Relaxer.

Fig. 1b. Micrograph of *Candida* spp (R1FB) isolated from Hair Relaxer

Fig. 1c. Micrograph of *Aspergillus glaucus* (R2FA) isolated from Hair Relaxer

Fig. 1d. Micrograph of *Cladosporium* spp (R3FA) isolated from Hair Relaxer.

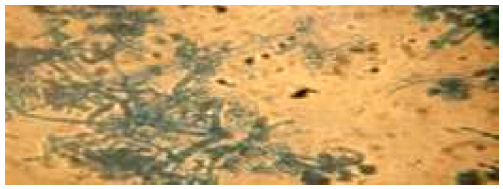

Fig. 1e. Micrograph of *Cladosporium* spp (R4FA) isolated from Hair Relaxer

Fig. 1f. Micrograph of *T. herzianum* (R4FB) isolated from Hair Relaxer

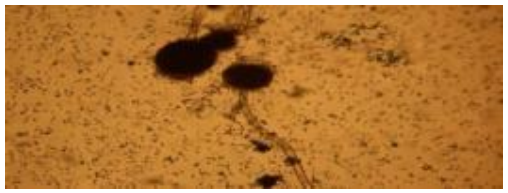

Fig. 1g. Micrograph of *A. niger* (LG1A) isolated from Lip Gloss

Fig. 1h. Micrograph of *Penicillium* spp (LG2A) isolated from Lip Gloss

Fig. 1i. Micrograph of *A. flavus* (LG2B) isolated from Lip Gloss

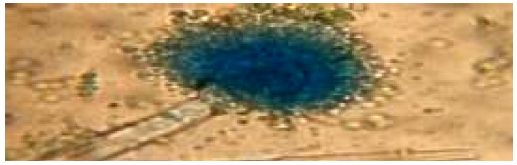

Fig. 1j. Micrograph of *A. flavus* (LG2C) isolated from Lip Gloss

Fig. 1k. Micrograph of *A. nidulans* (P1FA) isolated from Baby Lotion

Fig. 1l. Micrograph of *Penicillium* spp (P1FB) isolated from Baby Lotion

Fig. 1m. Micrograph of *Penicillium* spp (P2FA) isolated from Baby Lotion

Fig. 1n. Micrograph of *A. nidulans* (P2FB) isolated from Baby lotion

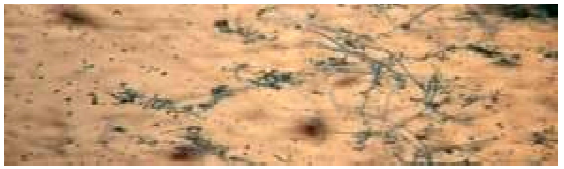

Fig. 1o. Micrograph of *Trichoderma* spp (P2FC) isolated from Baby Lotion

Fig. 1p. Micrograph of *C. albicans* (P3FA) isolated from Baby Lotion

Fig. 1q. Micrograph of *Rhizopus* spp (P3FB) isolated from Baby Lotion

Fig. 1r. Micrograph of *A. flavus* (P4FB) isolated from Baby Lotion

Fig. 1. Microscopic morphologies of the detected fungi from commercial cosmetic products

and other personal care products between the year 2004 and 2011 as reviewed by Scott and Jimenez [33], were similarly identified in this study. In addition, *Klebsiella pneumoniae*, *Penicillium* and *Aspergillus* were detected in the tested products thereby lending credence to the earlier reports of Osungunna et al. [14] and Okeke and Lamikanra [34]. However, contrary to the report of Razooki et al. [35], *Staphylococcus* and fungal contaminants were readily isolated from this present study, and this report provide a comprehensive knowledge on the diversity and characteristics of the fungal and bacterial contaminants, and potential pathogens associated with commercial cosmetic products. Considering the antibiogram of the detected bacteria, this present work is in tandem with the previous report of Gopalkrishna et al. [36]. *Serratia rubidaea* was resistant to Nitrofurantoin, Nalidixic acid, Colistin, Ampicillin and Streptomycin (Table 3).

The resistance pattern observed in the *S. rubidaea* is attributable to inducible chromosomal beta lactamase of the cephalosporinase type, thereby engendering resistance to Ampicillin and Cephalotin. The observed resistance by the bacteria isolates could be plasmid mediated or induced by the biocides that were employed in the sanitation of the factory equipment as well as the preservatives in the products. In many cosmetics, preservatives may loose activity [37], and such product becomes a potential risk for microbial contamination [38]. In this study, the observed high level of contaminants may signify the failure of the incorporated preservatives to inhibit the contaminants, as earlier indicated [39]. These findings imply that the cosmetics do not meet the stipulated microbiological quality standards in official monographs and can therefore serve as vehicles for the transmission of the detected pathogenic organisms. The products constitute potential health risks to unsuspecting consumers, and the manifestation of the risks are highly probable, considering the immune fragility of babies, inadvertent ingestion of microbially tainted ‘‘Lip gloss’’, and the potential inoculation of pathogens, subsequent upon possible skin breaches and the in-use chemical tenderization or “burns” of the adult’ scalp.

4. CONCLUSION

The isolation of *Buttiauxella agrestis*, which had not been previously reported, in addition to *Enterobacter gergoviae, Pseudomonas aeruginosa, Staphylococcus aureus, Aspergillus flavus, Penicillium* spp, and *Candida albicans* (designated as objectionable microorganisms in cosmetics) indicated that the cosmetics do not meet the stipulated microbiological quality standards in official monographs and can therefore serve as vehicles for the transmission of the detected pathogenic organisms. Therefore, Good Manufacturing Practice (GMP) should be strengthened, the efficacy, and continued use of the adopted preservatives should be reviewed, to ensure wholesomeness of the products through their shelf life.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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