Nig. J. Biotech. Vol. 34 (2017): 55-61
ISSN: 0189 1731
Available online at
http://www.ajol.info/index.php/njb/index
and www.biotechsocietynigeria.org
DOI: https://dx.doi.org/10.4314/njb.v34i1.7

Microbiological quality assessment of facial cosmetics

***Stanley, O. H[1], Immanuel, M. O[1], and Ekanem, P.[2]**

[1]Department of Microbiology, Faculty of Science, University of Port Harcourt, P. M. B. 5323, Choba, Port Harcourt, Rivers State, Nigeria.
[2]Department of Microbiology Technology, School of Science Laboratory Technology, University of Port Harcourt.

Abstract

Cosmetics are beauty enhancing agents for which microbial quality concerns have been neglected by users who often see them as innocuous. The aim of this study was to investigate the microbiological quality of selected facial cosmetic products. Thirty (30) samples each of in-use and unused cosmetics were obtained from users and cosmetics shops in Port Harcourt, Nigeria. The cosmetics sampled were lipsticks, eye shadows and foundations. The aerobic plate count and enrichment test methods were employed to isolate, enumerate and identify microbial contaminants using conventional presumptive and phenotypic identification methods. Microbial contaminants were present in 80% of the in-use cosmetics and 46.7% in the unused cosmetics. Results revealed presence of *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus* spp., *Pseudomonas aeruginosa, Candida albicans, Aspergillus* spp. and *Penicillium* spp in the in-use cosmetics and *Staphylococcus aureus, Staphylococcus epidermidis, Aspergillus* spp. and *Rhizopus* spp. in the unused cosmetics. Results revealed higher levels of contamination for in-use cosmetics with both pathogenic and non-pathogenic microbial species compared to the unused cosmetics. Results also indicated the failure of added preservatives to effectively inhibit microbial load to acceptable levels. Cosmetic use is on the increase and this study exposes the risk of using contaminated cosmetics products.

Keywords: Cosmetics, pathogenic, microbial load

*Correspondence: herbert.stanley@uniport.edu.ng

Introduction

Cosmetics are beauty articles or preparations which can be rubbed, poured, sprayed or placed in contact with the human body, with the intension to enhance aesthetic morphological appearance and improve conditions (Mwambete et al., 2009; Noor et al., 2015). Cosmetics are made from a range polymers and additives including proteins, carbohydrates, lipids, surface active agents, polymers, vitamins, absorbents, dyes and fragrances (Pinon et al., 2007; Jagessar et al., 2008). These cosmetics formulations and the abundance of water and other physical conditions, make them suitable media for microorganisms (Budecka and Kunicka-Styczyńska, 2014).

Cosmetic contaminants may gain access incidentally during the manufacturing or during use by consumers. Contamination of cosmetics by microorganisms may cause spoilage of the product and may present a serious health risk when pathogens are present (Rana et al., 2014). As a consequence, contamination may impact on the health of users and bring about economic losses to the producers (Zhang et al., 2009).

Most cosmetics are not sterile and they are made of non-sterile raw materials. Though cosmetic do not have to be sterile, microbial limit values have been reported according to the type of the cosmetics (Onurdað et al., 2010; Budecka and Kunicka-Styczyńska, 2014). Good manufacturing

and regulatory practices ensure that cosmetics entering the market are safe. Despite all efforts to improve microbiological quality of cosmetics, reports continue to appear in scientific literatures, suggesting high contamination (Babalola and Eze, 2015; Gamal et al., 2015; Skowron et al., 2017).

Cosmetics such as lipsticks, eye shadows and foundations are common women beauty products. Their safety to users because of the area of application necessitates microbial quality assessment. Facial cosmetics containing pathogens can pose serious health risk to the consumer due to the application area and ease of access to the eye nose and oral cavity (Budecka and Kunicka-Styczyńska, 2014). Parabens and other chemical additives are often added to cosmetics to prevent spoilage and to shield consumers against dangerous bacteria. It is obvious from available literatures that these additives are either not effective or not added in the right quantity (Onurdað et al., 2010; Mwambete and Simon, 2010). The aim of this study was to carry out the microbiological risk assessment of in-use and unused cosmetics.

Materials and Methods

Sample collection

A total of thirty (30) samples each of eye shadows, lipsticks and foundations, representing five different brands were used in this study. For each cosmetic sample used, 15 representing in-use samples were randomly collected from female students resident in the University of Port Harcourt hostels and the other 15 representing unused samples were bought from the open market in Port Harcourt, Nigeria. All samples had manufacturer's address, manufacturing date, batch number, NAFDAC number and were within their expiry dates.

Determination of Bacterial Contaminants

One gram (1 g) mass of sample was dispensed in 4 ml sterile Ringer solution containing 0.25% tween 80 and thoroughly mixed. The mixture was serially diluted with peptone water and a millilitre from 10^{-4} - 10^{-5} concentrations was plated out on nutrient agar, mannitol salt agar, blood agar, eosin methylene blue agar plates. All the plates were incubated at 37 °C for 48 hours. Colonies formed were counted and the mean count was calculated and expressed as colony forming units per gram (cfu/g) of products. The bacterial isolates were identified using Gram stain, oxidation fermentation, oxidase, and catalase tests described in the Bergey's Manual of Systematic Bacteriology.

Determination of Fungal Contaminants

One (1) ml of dilutions (10^{-2}-10^{-3}) was inoculated on Sabouraud dextrose agar (SDA) plates using pour plate method. The plates were then incubated at 25°C for 5 days. Enumeration of colonies was expressed as yeasts and moulds count per gram. Fungal isolates were identified based on their macroscopic and microscopic characteristics. Macroscopic identification was done by visualizing surface pigment and reverse pigment on SDA plates and microscopic characterization includes shape, color and structure of conidia, hyphae, conidiophores and conidial head.

Determination of Moisture

Moisture was determined by weighing petri-dish dried in an oven at 105°C for 3 hours and another containing 3 g of samples dried at same temperature until a constant weight was achieved. The difference in mass was taken as the moisture content.

Determination of pH

The pH was determined using a pH meter (GLP 21 (Crison Instruments, S.A., Barcelona, Spain) after homogenizing 1g of the sample in 10 ml of distilled water to form slurry in which the pH meter was inserted.

Results

Results for microbial content test for tested cosmetics revealed presence of *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus* spp., *Pseudomonas aeruginosa, Candida albicans, Rhizopus* spp. *Aspergillus* spp. and *Penicillium* spp. as contaminants of the tested cosmetics (Table 1). The microbial diversity was more in the in-use cosmetic than the unused.

Table 1: Microbial contaminants of facial cosmetics

Cosmetic	In-use		Unused	
	Bacteria (cfu/g)	Fungi (cfu/g)	Bacteria (cfu/g)	Fungi (cfu/g)
Eye shadow-1	*Staphylococcus aureus* and *S. epidermidis*	ND	*S. aureus*	ND
Eye shadow-2	*S. aureus*	ND	*S. aureus*	ND
Eye shadow-3	ND	ND	ND	ND
Eye shadow-4	ND	ND	ND	ND
Eye shadow-5	*S. aureus*	ND	ND	ND
Lipstick-1	*Streptococcus* spp.	*Candida albicans*	ND	*Rhizopus* spp.
Lipstick-2	*S. aureus*	ND	ND	ND
Lipstick-3	*S. aureus*	ND	ND	ND
Lipstick-4	*Streptococcus* spp. and *Pseudomonas aeruginosa*	ND	ND	ND
Lipstick-5	*Streptococcus* spp.	ND	ND	ND
Foundation-1	*S. aureus* and *S. epidermidis*	*Aspergillus* spp. and *Penicillium* sp.	*S. aureus*	ND
Foundation-2	*S. aureus*	ND.	*S. aureus*, and *S. Epidermidis*	ND
Foundation-3	*S. aureus*	ND.	ND	*Aspergillus* spp.
Foundation-4	*S. aureus*	ND	ND	ND
Foundation-5	*S. aureus*	*Aspergillus* spp.	*S. aureus*	ND

ND: Not detected

Table 2 shows the microbial counts and physical properties of cosmetic products. The microbial counts ranged from $1.0x10^2$ - $4.0x10^2$ cfu/g and $6.0x10^2$ - $3.0x10^4$ cfu/g for unused and in-use cosmetics respectively. The counts for the in-use eye shadow ranged from $2.0x10^4$ - $7.2x10^3$ cfu/g; $6.0x10^2$ $3.0x10^4$ cfu/g for lipstick and $1.0x10^3$ $3.0x10^3$ cfu/g for foundation. There was no fungal contaminant in eye shadow for both in-use and unused cosmetics. The pH values were moderately acidic or alkaline. Moisture content ranged from moderate to high.

Table 2: Microbial counts and physical properties of cosmetic products

Cosmetic	In-use				Unused			
	Aerobic Plate Count				Aerobic Plate Count			
	Bacteria (cfu/g)	Fungi (cfu/g)	pH	Moisture (%)	Bacteria (cfu/g)	Fungi (cfu/g)	pH	Moisture (%)
Eye shadow-1	7.0×10^3	-	6.8	0.71	1.0×10^2	-	8.3	0.73
							7.4	0.56
Eye shadow-2	2.0×10^3	-	7.2	0.57	4.2×10^2	-		
Eye shadow-3	4.3×10^3	-	7.8	0.66	2.6×10^2	-	7.0	0.57
Eye shadow-4	7.2×10^3	-	8.1	0.62	1.0×10^2	-	6.8	0.64
Eye shadow-5	3.5×10^3	-	7.2	0.53	3.0×10^2	-	6.3	0.52
Lipstick-1	$6.0x10^2$	$2.0x10^2$	5.8	0.67	-	$1.0x10^2$	5.0	0.68
Lipstick-2	3.0×10^4	-	7.5	0.66	-	-	8.2	0.65
Lipstick-3	$1.2x10^3$	-	8.1	0.66	-	-	6.4	0.68
Lipstick-4	$1.4x10^3$	-	7.0	0.74	-	-	5.5	0.62
Lipstick-5	$2.2x10^3$	-	7.4	0.64	-	-	6.8	0.60
Foundation-1	$3.0x10^3$	$2.2x10^2$	6.1	0.77	$1.0x10^2$	-	6.4	0.62
Foundation-2	$1.0x10^3$	-	5.8	0.70	$2.0x10^3$	-	7.2	0.67
Foundation-3	$5.0x10^3$	-	6.2	0.68	-	$1.0x10^2$	5.8	0.70
Foundation-4	$3.0x10^3$	-	5.8	0.65	-	-	6.4	0.62
Foundation-5	$2.8x10^3$	$1.0x10^2$	4.8	0.69	$2.0x10^2$	-	7.1	0.73

Discussion

Microbiological quality assessment of selected facial cosmetic products revealed *Staphylococcus* spp. and *Pseudomonas aeruginosa* as the prevalent isolates. *Staphylococcus* spp. and *Pseudomonas aeruginosa* have been reported in several studies as the most commonly isolated bacteria from cosmetic products (Lundov and Zachariae, 2008; Mwambete and Simon, 2010; Gamal et al., 2015). *Pseudomonas aeruginosa* is an opportunistic pathogen capable of causing infections in immune-compromised consumers (Budecka and Kunicka-Styczyńska, 2014). *Staphylococcus aureus* is capable of causing serious skin infections (Begier et al., 2004). *Candida albicans* is notorious for superficial mycotic infections in normal and immune-compromised persons (Mierzejewski, 2013; Babalola and Eze, 2015). *Aspergillus* spp. and *Penicillium* spp. are among the fungi most frequently isolated from cosmetics for which concerns of infections in immune-compromised individuals have been reported (Babalola and Eze, 2015). *Rhizopus* spp. can also cause opportunistic skin infection (Ibrahim et al., 2012). The presence of pathogenic and opportunistic microorganisms in cosmetic products is undesirable, as it leads to changes in texture, colour, odour and general loss in integrity of products.

Cosmetics can provide the physical and chemical environment for microorganisms to grow and thrive. The moisture contents of tested cosmetics were within 0.52 0.77 %. The amount of available water in cosmetic formulations will affect microorganism's growth and survival by affecting generation time and metabolic activities. All tested cosmetics had pH values ranging from 4.8 8.2, which are suitable for bacterial and fungal metabolism (Table 1). Cosmetic formulations vary but do include nourishing and easily metabolized ingredients such as lipids, proteins, vitamins and minerals (Pinon et al., 2007; Jagessar et al., 2008). Hence, they could support microbial lives.

Regulatory agencies have microbial content limits for cosmetic products (EEC, 1976; FDA, 2004). For some products, it has been specified that objectionable microorganisms be absent, while for others, excessive number of microbial deteriogens are prohibited. Absence of known pathogens and opportunistic pathogens in cosmetic products is statutory according to ISO 22716. *Pseudomonas aeruginosa*, *Candida albicans* and *Staphylococcus aureus* which are potentially pathogenic microorganisms were present in the studied cosmetic products. Lipstick had species of all the three pathogens of concern while eye shadow and foundation had only species of *Staphylococcus aureus*.

In the present study *Staphylococcus* spp. which is part of the normal human microflora, were the most isolated from the cosmetic products in-use and unused. Their presence in unused samples could be due to poor manufacturing practices, inadequacy or ineffectiveness of added preservative, storage and transport conditions. Presence of *Staphylococcus* spp. in in-use cosmetic is not unexpected but when values are excessive, it calls for concern. Staphylococcal infection is a major risk particularly by methicillin-resistant *Staphylococcus aureus* (Begier et al., 2004)

The microbial counts for unused cosmetic were between $1.0x10^2$ - $4.0x10^2$ cfu/g which is within the recommended ISO 17516 cosmetic microbiology microbiological limits for non-objectionable microorganisms in eye-area products not meant for babies (< 500 cfu/g). The counts for the in-use eye shadow ranged between $2.0x10^4$ and $7.2x10^3$ cfu/g. These values far exceeded the limit for eye-area products, reaching critical threshold capable of causing infection (Onurdað et al., 2010; Mierzejewski, 2013). Based on the obtained results concerning cosmetics in-use (Table 2-4), it was observed that 80% of the tested in-use cosmetics have contaminants exceeding the acceptable limits. Of the sampled unused cosmetics, 46.6% showed presence of microorganisms. Gamal et al. (2015) reported in their study that commercial cosmetic creams did not generally meet the standards for microbial limits. Although the microbial counts in unused cosmetic were low, the presence of *Staphylococcus aureus* is objectionable. The bacteria contaminants were more in number than fungi in this study. This agrees with other studies reported in the literatures (Gamal et al., 2015; Skowron et al., 2017).

Conclusions

The present study confirms that microbiological quality of cosmetic products is one that needs to be given more attention as the use of cosmetic products is on the rise. The in-use cosmetics were grossly contaminated; arising from prolong exposure and handling of the products, as indicated by the presence of microorganisms which are part of normal human microflora. The presence of pathogenic and opportunistic microorganisms in beauty products raises doubt about their overall safety and consumers should be aware of the risk.

References

Babalola, M. O. and Eze, M. (2015). Microbiological quality and characterization of potential pathogens associated with selected brands of commercial cosmetic products in Nigeria. *British Microbiology Research Journal*. 9(5): 1 - 17.

Begier, E. M., Frenette, K., Barrett, N. L., Mshar, P., Petit, S., Boxrud, D. J., Watkins-Colwell, K., Wheeler, S., Cebelinski, E. A., Glennen, A., Nguyen, D. and Hadler, J. L. (2004). A high-morbidity outbreak of methicillin-resistant *Staphylococcus aureus* among players on a college football team, facilitated by cosmetic body shaving and turf burns. *Clin Infect Dis*. 39(10): 1446-53. doi: 10.1086/425313.

Budecka, A. and Kunicka-Styczyńska, A. (2014). Microbiological contaminants in cosmetics isolation and characterization. *Biotechnol* Food Sci. 78 (1): 15 - 23.

European Economic Community, EEC (1976). Council Directive of 27 July 1976 on the approximation of the Laws of the Member States relating to cosmetic products 76/768/EEC. *Off J Eur Comm* 1976, No. L 262/170.

Food and Drug Administration, FDA (2004). The Federal Food and Drug Cosmetic Act Criteria. Retrieved from http://www.fda.gov/opacom /laws/fdcact/fdctoc.htm, 09:12: 2015.

Gamal M. A. B., Abo Azza M. M., Al Gayeed, A. O. A. and Sawan, M. S. (2015). Microbiological Quality Assessment of Some Brands of Cosmetic Creams Sold Within Alkhoms City, Libya. *IOSR Journal of Dental and Medical Sciences*. 14: (2) 60 - 65.

Ibrahim, A. S., Spellberg, B., Walsh, T. J. and Kontoyiannis D. P(2012). Pathogenesis of mucormycosis. *Clinical Infectious Diseases*. 54: 16 - 22.

ISO 22716 (2009). Cosmetics Good Manufacturing Practices (GMP).

ISO 17516 (2014). Cosmetic Microbiology Microbiological Limits

Jagessar., R. C. Mars, A. and Gones, G. (2008). Selective antimicrobial properties of leaf extract against various microorganisms using disc diffusion and agar well diffusion method. *J. Nat Sci.* 6:24 - 38.

Lundov, M. D. and Zachariae, C. (2008). Recalls of microbiologically contaminated cosmetics in EU from 2005 to May 2008. *Int J Cosmetic Sci*. 30: 471 - 474.

Mierzejewski J. (ed.). (2013). Cosmetics microbiology. Uniwersytet Technologiczno-Humanistyczny im. Kazimierza Pułaskiego, Radom, Poland. pp. 166 - 219.

Mwambete, K. D. and Simon, A. (2010). Microbiological quality and preservative capacity of commonly available cosmetics in Dar es Salaam, Tanzania East and Central *African Journal of Pharmaceutical Sciences*. 13: 3- 11. the presence of ethanol. Int J Cosmetic Sci. 29: 111 - 119.

Mwambete, K. D., Justin-Temu, M. and Fazleabbas, F. S. (2009). Microbiological assessment of commercially available quinine syrup and water for injection in Dar Es Salaam, Tanzania. *Trop J Pharm Res*. 8(5): 441 - 447.

Noor, R., Zerin, N. and Das, K. K. (2015). Microbiological quality of pharmaceutical products in Bangladesh: current research perspective. *Asian Pac J Trop Dis*. 5: 264 - 70.

Onurdað, F. K., Özgen, S. and Abbasoðlu, D. (2010). Microbiological investigation of used cosmetic samples. *Hacettepe University Journal of the Faculty of Pharmacy*. 30 (1): 1 - 16.

Pinon, A., Alexandre, V., Cupferman, S., Crozier, A. and Vialette, M. (2007). Growth, survival and inactivation of *Pseudomonas aeruginosa* and *Staphylococcus aureus* strains of various origin in the presence of ethanol. *Int J Cosmetic Sci*. 29: 111 - 119.

Rana. J. Sultana, T., Das, K. K. and Noor, R. (2014). Microbiological analysis of topical available in Bangladesh. *Int J. Pharm. Pharma*. Sci. 6: 330 - 342.

Skowron, K., Jakubic, A., Budzyńska, A., Kaczmarek, A., Grudlewska, K., Reśliński, A. and Gospodarek-Komkowska, E. (2017). Microbiological purity assessment of cosmetics used by one and several persons and cosmetics after their expiry date. *Rocz Panstw Zakl Hig. 68* (2): 191 - 197.

Zhang, W., Lu, B., Guo, R. and Chen, X. (2009). Study on hygienic microbe pollution for imported cosmetics during 2003-2006. *Chinese Journal of Health Laboratory Technology.* 3: 79 - 87.