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Evaluation of Bacteriological Quality and Preservatives Efficacy of Cosmetics

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Abstract **Original Research Article**

Cosmetics are external preparations normally applied to human body parts to enhance or alter the appearance of the face or fragrance and texture of the body. The common cosmetic products include: foundations, mascaras, powders, lipsticks, eye shadow, skin cleansers, body lotions, shampoos, hairstyling products (gel, hair spray, etc.), perfumes and colognes. Total of (96) cosmetic products were investigated. Twenty (20) samples of foundations, mascaras, compact powders, (5) samples of lip-gloss and (15) sponges of compact powder making a total of 80 products were bought from the market and evaluated for their bacteriological quality before use (as new products) and after three months of their use by volunteers. Total bacterial count was carried out using pour plate technique, and then all bacterial isolates were identified using microscopic examination, biochemical reactions, and gram staining technique. These identifications were confirmed by the use of Analytical Profile Index (API). Challenge test was conducted on (8) foundations and (8) compact powders of different brands to determine the efficacy of preservative(s) included in their formulations. The results revealed that only 15 (18.75%) out of 80 cosmetic products were found contaminated with bacteria and fungi after use. The maximum bacterial contamination (40%) was observed in lip-gloss samples, followed by 35%, 25% and 0.6% of contamination for mascara, foundation and sponge samples respectively. In contrast no bacterial contamination was detected in compact powder samples. The bacterial viable count of 15 cosmetic products showed that most of them exhibited bacterial count ranging between 2.37 x 10^{-5} and 2 x10^{-4} CFU. High viable bacterial count (2 x10^{-4}) was observed in mascara samples of *Mac* trademark. The predominant bacterial isolates were *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The *S. aureus* was predominant in both lip-gloss and foundation samples followed by *Klebsiella pneumonia* in mascara and sponge samples. The results of challenge test emphasized that, according to United States Pharmacopoeia (USP) legislation standard products of foundation of *Maxfactor*® and compact powder of *Dermacool*® were accepted, in which their preservatives were able to inhibit the growth and kill all inoculated standards of both bacteria and fungi after 14 and 28 days of incubation. However, the other reaming cosmetic products were able to inhibit the growth and kill the inoculated standard of bacteria after 14 and 28 days but unable to suppress or inhibit the growth of *Candida albicans* after 28 days of incubation time. In conclusion, according to USP legislation standard the tested cosmetic products unacceptable and considered as rejected products.

Keywords: Cosmetics, Cosmetics Contamination, Microbial Contamination, Challenge Test.

INTRODUCTION

In the United States, the Food and Drug Administration (FDA), which regulates cosmetics defines cosmetics as products "intended to be applied to the human body for cleansing, beautifying, promoting attractiveness, or altering the appearance without affecting the body's structure or functions". This broad definition includes any material intended for use as an ingredient of a cosmetic product, with the FDA specifically excluding pure soap from this category and European commission (EC) regulation defines cosmetics as (any substance or mixture intended to be placed in contact with the external parts of the human body (epidermis, hair, nails, lips…etc.) or with the teeth and mucous membranes of the oral cavity with the exclusive or principal objective to clean, perfume, or protect them or, changing their appearance or keeping them in good condition. (Chaudhri and Jain, 2009; Guillerme *et al.*, 2017). Depending on the application area, cosmetics may be categorized as cosmetics for skin, hair-scalp and oral care as well as fragrances (Mitsui, 1997).

The estimated growth rate of global cosmetics market was 6.4% in the year 2020. The global cosmetics market was $460 billion in 2014 and was expected to

reach $675 billion by 2020 (Wood, 2018). Nowadays, microbial contamination and monitoring toxic ingredients as continuous multidimensional control are required in this rising market of cosmetics. Before 1930s there wasn't any importance about studying cosmetics and microbiology but it becomes more important in 1940s (Curry *et al*., 2006).

Sterility is not a main issue for cosmetics to be accepted but they have to be free from pathogenic organisms like *S. aureus, E. coli, P. aeruginosa* and low levels of the total aerobic microbial count. The presence of pathogens and high levels of organisms in cosmetic products can cause spoilage in the form of physical deterioration of the products and pose a high risk for consumer's health (Becks and Lorenzoni, 1995; Behravan *et al*., 2005; Campana *et al*., 2006). For the last three decades Good Manufacturing Practices (GMP) has been implemented as a good strategy to improve industrial quality control analyses. In support to this purpose, Microbial Limits Test has been established according to the USP to determine total microbial count for bacteria, yeast and mould using several methods of analyses (Anon, 2006). Despite of these regulations microbial contamination is still playing a major role in product recalls worldwide, especially in developing countries (Okeke and Lamikanra, 2001). Therefore, improving preservative system (Farrington *et al*., 1994; Linter and Genet, 1998) is a good way to inhibit growth of organisms during manufacturing, storage and handling by consumers, also the use of non-invasive packaging is recommended (Brannan and Dille, 1990). During the years between 2008 and 2014, many cosmetic products were recalled from 14 different countries due to microbial contamination and the number of countries that recalled cosmetics from the market was raised in 2013 and 2014 (Neza, 2016). On other hand, the presence of microorganisms in cosmetic products and their exposure to atmospheric oxygen are capable of causing modification to cosmetic products. Generally, addition of antimicrobial preservatives to prevent microbial spoilage of cosmetic products and addition of antioxidant preservatives to prevent oxidation phenomena and preventing the release of free radicals are two very important measures renders cosmetic products very safe (Martini, 2006).

Therefore, in accordance with the legislation in force, this study aimed to evaluate the possible bacterial contamination of several cosmetic products in two different ways of their use (before and after-use). Moreover, evaluating the efficacy of preservatives included in these cosmetic formulations using microbiological challenge test.

OBJECTIVE

This study assessed and verifies the bacteriological quality of some brands of cosmetic products marketed in the country and sold within the city of Tripoli, Libya in two different ways of their use (before-use and after-use). Moreover, evaluating the efficacy of preservatives included in these cosmetic formulations using microbiological challenge test.

METHODS

Setting and Study Design

This study is a prospective microbiological evaluation. The study was carried on five types of cosmetics namely; four foundations (*Mac®*, *Maxfactor®*, *Deborah® & Ever beauty®*), four mascaras (Mac®, Maxfactor®, *Maybelline®* & New well®), four compact powders (*L'Oreal®*, *Derma cool®*, *Maybelline®* & *Final touch®*), sponges of compact powder and lip-gloss. These products were taken as new (unopened) products and as (opened) products used by volunteers in city of Tripoli. Each volunteer was invited to informed consent.

Sample Collection

As shown in figure 1, a total of 96 cosmetic products were investigated. One hundred and sixty (160) samples were tested in two different ways of use, the intact product (at the time of purchase as new product), and the in-use product (after 3 months of use) were collected and investigated in order to verify the degree of possible bacteriological contamination during their use by volunteers. Twenty (20) samples of each of cosmetic products of different brands and packages namely; Foundations, mascaras, and compact powders. In addition to 5 lip-gloss and 15 sponges of compact powder, making a total of 80 samples were taken from commercially cosmetics available in the Libyan market and sold as new (unopened) products and tested before and after three months of volunteer's use. All samples were taken to the laboratory of microbiology in the department of microbiology and immunology at faculty of pharmacy, university of Tripoli to evaluate their bacteriological quality using standard microbiology procedures. Lastly, 8 samples of each of foundations and compact powders (total of 16 samples) were taken and investigated for the efficacy of their included preservative(s) using challenge test.

MICROBIOLOGICAL ANALYSIS

Bacterial Identification

Small amount samples of each of foundation, compact powder and lip-gloss were transferred to 9ml of phosphate buffer solution containing 0.5% of tween 80 and small amount of mascaras samples were transferred to 9ml of phosphate buffer containing 0.5% of tween 20, while small pieces of sponges were transferred to 9ml of nutrient broth then incubated at 37ºC for 24 hrs then inoculated in the following selective media: MacConkey agar (MCA) is selective for gram negative organisms and helps to differentiate lactose fermenting gram-negative rods from non- lactose fermenting gram-negative rods. It is primarily used for the detection and isolation of members of family *Enterobacteriaceae* and *Pseudomonas* spp. Mannitol salt agar (MSA) is a selective, differential and indicator medium which was used to isolate and identify *S. aureus*.

Salmonella-Shigella (SSA) agar is a selective and differential medium was used to isolate *Salmonella* and *Shigella*. Muller Hinton Agar (MHA) and Sabouraud dextrose agar (SDA) were used for the isolation and cultivation of fungi and yeasts respectively.

Routine microbiological tests; gram staining, biochemical tests (*catalase test*, *coagulase test*, *triple sugar iron agar test*, *indole test*, *oxidase test*) were performed on isolated colonies and then confirmed by using API kits.

Total Bacterial Count

The collected samples of cosmetic products were investigated for the determination of total viable bacterial count (before and after use) using pour plate method. Each sample, 10-fold serially diluted in physiological buffer and opportunely homogenized, and was spread on plate count agar (PCA; Himedia, Mumbai, India) and selective agar according to the founded contaminant and incubated at 37ºC. The plates were observed after 24-48 hrs and the number of colony-forming units (CFU/ml) was determined. Each assay was performed in duplicate.

Challenge Test for Preservative Efficacy

A total of 16 cosmetic products (8 foundations and 8 compact powders) were chosen and subjected to challenge test (figure 1). Each cosmetic product was divided into 4 groups. Each group contains two of foundation or compact powder with the same type of preservatives. One preservative was taking from each group, making a total of eight different preservatives were tested for their efficacy using challenge test (figure 1). The challenge test for efficacy of antimicrobial preservation of cosmetic products was investigated as suggested by USP. Reference strains of *P. aeruginosa* ATCC 25850, *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *C. albicans* ATCC 10231 were obtained from department of microbiology, faculty of medicine, university of Tripoli, Tripoli, Libya. Freshly grown bacteria and fungi were prepared in a concentration of 1×10^{-8}cfu/ml. All inoculated samples were incubated at 25ºC for 28 days. Two grams of aseptic samples were removed on days 0, 14, and 28 and added to the neutralizing medium, and the total viable microbial count was determined by plate count using (PCA) and selective media for each inoculated organism. Plates were incubated for 24-48 hrs at 37°C for bacteria and at 25°C for 5 days for fungi. At the end of the incubation period, the number of colonies was recorded for each plate and counts were expressed as colony forming units per gram (cfu/g). The acceptance criteria for bacteria was at least the second logarithmic reduction from initial count and no increase from the 14 days count to 28 days and for fungi no increase from the initial count at 14 and 28 days (USP, 2003; Birteksoztan *et al*., 2013).

STATISTICAL ANALYSIS

All experiments were carried out in triplicate (3 biological replicas and 3 technical replicas). The results are presented as means ±SD. Statistical analysis was conducted using the SPSS version20. After assumptions of normality, the variances of homogeneity were checked by one-way analysis of variance (ANOVA) test and Chi- square test were performed on the data. The significance level was set at $p<0.05$.

RESULTS

Samples from un-opened (new) compact powder, foundation, lip-gloss and sponge products showed no microbial growth. On the other hand, after performing gram staining on each of five samples of un-opened (new) *Maybelline*® Mascara, only one sample revealed growth of *P. aeruginosa* which was then confirmed by *oxidase* and *TSI* tests. Regarding detection of microbial contamination after three months of use the results shown in table 3 revealed that only 15 out of 80 (18.75%) cosmetic samples were found contaminated with bacteria and fungi. The maximum bacterial contamination (40%) was observed in lip-gloss samples, followed by 35%, 25% and 0.6% of contamination for mascara, foundation and sponge samples respectively. In contrast no bacterial contamination was detected in compact powder samples. the statistical analysis carried out by Chi-square test revealed that, the difference in contamination with lip - gloss, mascara and foundation before and after volunteer's use was statistically significant with a *p*-value less than 0.05 (< 0.05), However, there was no significant difference in the use of compact powders and their sponges with a *p*-value more than 0.05 (> 0.05). Furthermore, the use of lip-gloss showed higher contamination incidence (40%) than did the mascara (35%), then followed by foundation (25%). The lowest degree of contamination (0.6%) and (0%) was observed for compact powder's sponge and compact powder itself respectively and statistically different in contamination with lip- gloss, mascara and foundation this statistically difference means there is strong relation between the use of these products and their contamination.

The result in table 4 represents total microbial viable count for different cosmetic products. The microbial evaluation of 15 cosmetic products showed that most of them exhibited microbial count ranging between ($2.75\ x10^{-6}$ and $2\ x10^{-4}$) cfu/ml. High viable microbial count ($2\ x10^{-4}$) cfu/ml was observed in mascara sample of *Mac*®. The lowest microbial count ($2.75\ x10^{-6}$ cfu/ml) was observed in foundation samples of *New well*®. The total bacterial viable count was ranging between ($2.37\ x10^{-5}$ and $2\ x10^{-4}$ cfu/ml). High viable bacterial count ($2\ x10^{-4}$) cfu/ml was observed in mascara sample of *Mac*®. While the lowest bacterial count ($2.37\ x10^{-5}$) cfu/ml was observed in mascara of *Maybelline*®. Among 15 of the total investigated samples of used cosmetic products, *S. aureus* was predominant in both lip-gloss and foundation samples followed by *K.*

pneumonia in mascara of *Maybelline*® and sponge samples. The presence of *Rhizopus* spp. was only observed in foundation of *New well*® and mascara of *Maxfactor*® samples.

The effectiveness of preservatives using challenge test was performed on 16 cosmetic products (8 foundations and 8 compact powders). The results showing in table 5 emphasized that, all cosmetic products did not show sign of bacterial contamination during the total viable count using three stains of reference bacteria; *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 25850. However, both foundation and compact powder samples yielded fungal contamination of *C. albicans ATCC* 10231 counts with preservative activity results ranging from 1.6 x 10^{-8} to 7.8 x 10^{-7} cfu/g after 28 days of incubation period. Whereas, no growth of *C. albicans* was observed for *Maxfactor*®, *Deborah*® and *Mac*® of foundations and *L'Oreal*® and *Derma cool*® of compact powders after 14 days of incubation time.

The results in table 5 clearly demonstrated that two cosmetic products namely; *Maxfactor*® of foundations (figures 1a) and *Dermacool*® of compact powders (figures 2a) out of 8 trademarks of cosmetic samples passed the preservative effectiveness test.

Table 1: Contaminated samples of un-opened (new) cosmetic products

COSMETICS TYPE	TRADEMARKS	CODE	SELECTIVE CULTURE MEDIA				
			MSA	MCA	SSA	MHA	SDA
Compact powder	N/A	N/A	-	-	-	-	-
Foundation	N/A	N/A	-	-	-	-	-
Lip-gloss	N/A	N/A	-	-	-	-	-
Mascara	Mac® (M.C.)	M.C.1	-	-	-	-	-
		M.C.2	-	-	-	-	-
		M.C.3	-	-	-	-	-
		M.C.4	-	-	-	-	-
		M.C.5	-	-	-	-	-
	Maxfactor® (M.X.)	M.X.1	-	-	-	-	-
		M.X.2	-	-	-	-	-
		M.X.3	-	-	-	-	-
		M.X,4	-	-	-	-	-
		M.X.5	-	-	-	-	-
	Maybelline® (M.B)	M.B.1	-	-	-	-	-
		M.B.2	-	-	-	-	-
		M.B.3	-	-	-	-	-
		M.B.4	-	-	-	+	-
		M.B.5	-	-	-	-	-
	New well® (M.N.)	M.N.1	-	-	-	-	-
		M.N.2	-	-	-	-	-
		M.N.3	-	-	-	-	-
		M.N.4	-	-	-	-	-
		M.N.5	-	-	-	-	-
Percentage (%) of contamination			0%	0%	0%	1%	0%

MSA, Mannitol salt agar; MCA, MacConkey agar; SSA, Salmonella-shigella agar; MHA, Muller Hinton agar; SDA, Sabouraud dextrose agar.

Table 2: Cosmetic products and their preservative's content

Cosmetic products		Types of preservative
Types of cosmetics	Trademarks	
Foundation	*Maxfactor*®	Sodium dehydroacetate Methyl paraben Propyl paraben
	Deborah®	Stearic acid Methyl paraben
	Ever beauty®	Phenoxyethanol Methyl paraben Butyl paraben
	Mac®	Phenoxyethanol

Cosmetic products		Types of preservative
Types of cosmetics	Trademarks	
		Sorbic acid
Compact powder	*L'Oreal*®	Methyl paraben Propyl paraben Ethyl paraben
	Derma cool®	Phenoxyethanol
	Maybelline®	Potassium sorbate Sodium dehydroacetate
	Final touch®	Propyl paraben Methyl paraben

Table 3: Detection of microbial contamination after the use of cosmetic products

Cosmetic product	Number of examined samples	Contaminated samples	
		Number	Percentage (%)
Lip-gloss	5	2	%40
Mascara	20	7	%35
Foundation	20	5	%25
Sponge	15	1	%0.6
Compact powder	20	0	%0
Total	80	15	18.75%

Table 4: Total microbial viable count and types of bacterial and fungal contaminants isolated from contaminated samples of cosmetic products after use.

Cosmetic products				Contaminated organisms	
Type	Trademarks	Contaminated samples			
		Number	Total	CFU / ml^{-1}	Isolated strain
Lip gloss	Forever®	1	2	TMTC	*S. aureus*
	Deborah®	1		$1.18x10^{-4}$	*S. aureus*
Foundation	Maybelline®	2	5	TMTC	*S. aureus*
				$1.15x10^{-4}$	*S. aureus*
	Mac®	1		$1.1x10^{-4}$	*S. aureus*
	New-well®	2		$1.3x10^{-4}$	*S. aureus*
				$2.75x10^{-6}$	*Rhizopus spp.*
Mascara	Maybelline®	4	7	$1x10^{-4}$	*K. pneumonia*
				$2.37x10^{-5}$	*K. pneumonia*
				$1.87x10^{-4}$	*K. pneumonia*
				TMTC	*P. aeruginosa & K. pneumonia.*
	Mac®	1		$2x10^{-4}$	*Enterobacter amnigenus*
	Maxfactor®	1		TMTC	*Rhizopus spp.*
	New-well®	1		$0.23x10^{-4}$	*S. aureus*
Sponge	Maybelline®	1	1	$0.9x10^{-4}$	*K. pneumonia*
Total examined cosmetic products			15		

TMTC, Too Many To Count.

Table 5: Preservatives efficacy by challenge test

Types of cosmetics	*Trademark*	Colony Forming Units (CFUs) / gram											
		***E. coli* ATCC 25922**			***S. aureus* ATCC 25923**			***P. aeruginosa* ATCC 25850**			***C. albicans* ATCC 10231**		
		0 day	**14 days**	**28 days**	**0 day**	**14 days**	**28 days**	**0 day**	**14 days**	**28 days**	**0 day**	**14 days**	**28 days**
Foundation	***Maxfactor*®**	$8.5 x 10^{-8}$	-	-	$6.4 x 10^{-8}$	-	-	$2.8 x 10^{-9}$	-	-	$5.1 x 10^{-8}$	-	-
	Deborah®	$1.2 x 10^{-9}$	-	-	$4.1 x 10^{-8}$	-	-	$3.7 x 10^{-8}$	-	-	$4.9 x 10^{-8}$	-	$5.3 x 10^{-8}$
	Ever beauty®	$1.7 x 10^{-9}$	-	-	$6.0 x 10^{-8}$	-	-	$2.4 x 10^{-9}$	-	-	$7.3 x 10^{-8}$	$1.8 x 10^{-8}$	$7.8 x 10^{-7}$

Types of cosmetics	***Trademark***	**Colony Forming Units (CFUs) / gram**											
		***E. coli* ATCC 25922**			***S. aureus* ATCC 25923**			***P. aeruginosa* ATCC 25850**			***C. albicans* ATCC 10231**		
		0 day	**14 days**	**28 days**	**0 day**	**14 days**	**28 days**	**0 day**	**14 days**	**28 days**	**0 day**	**14 days**	**28 days**
	Mac®	1.4×10^{-9}	-	-	7.8×10^{-8}	-	-	2.2×10^{-9}	-	-	6.0×10^{-8}	-	7.5×10^{-7}
Compact powder	*L'Oreal*®	2.1×10^{-9}	-	-	2.4×10^{-8}	-	-	7.4×10^{-8}	-	-	1.7×10^{-8}	-	3.0×10^{-8}
	Derma cool®	5.8×10^{-8}	-	-	2.0×10^{-8}	-	-	4.5×10^{-8}	-	-	1.4×10^{-9}	-	-
	Maybelline ®	3.0×10^{-8}	-	-	1.0×10^{-8}	-	-	1.1×10^{-8}	-	-	1.9×10^{-8}	1.5×10^{-9}	1.6×10^{-8}
	Final touch®	8.4×10^{-8}	-	-	1.0×10^{-8}	-	-	9.5×10^{-8}	-	-	1.8×10^{-8}	0.78×10^{-8}	0.98×10^{-7}

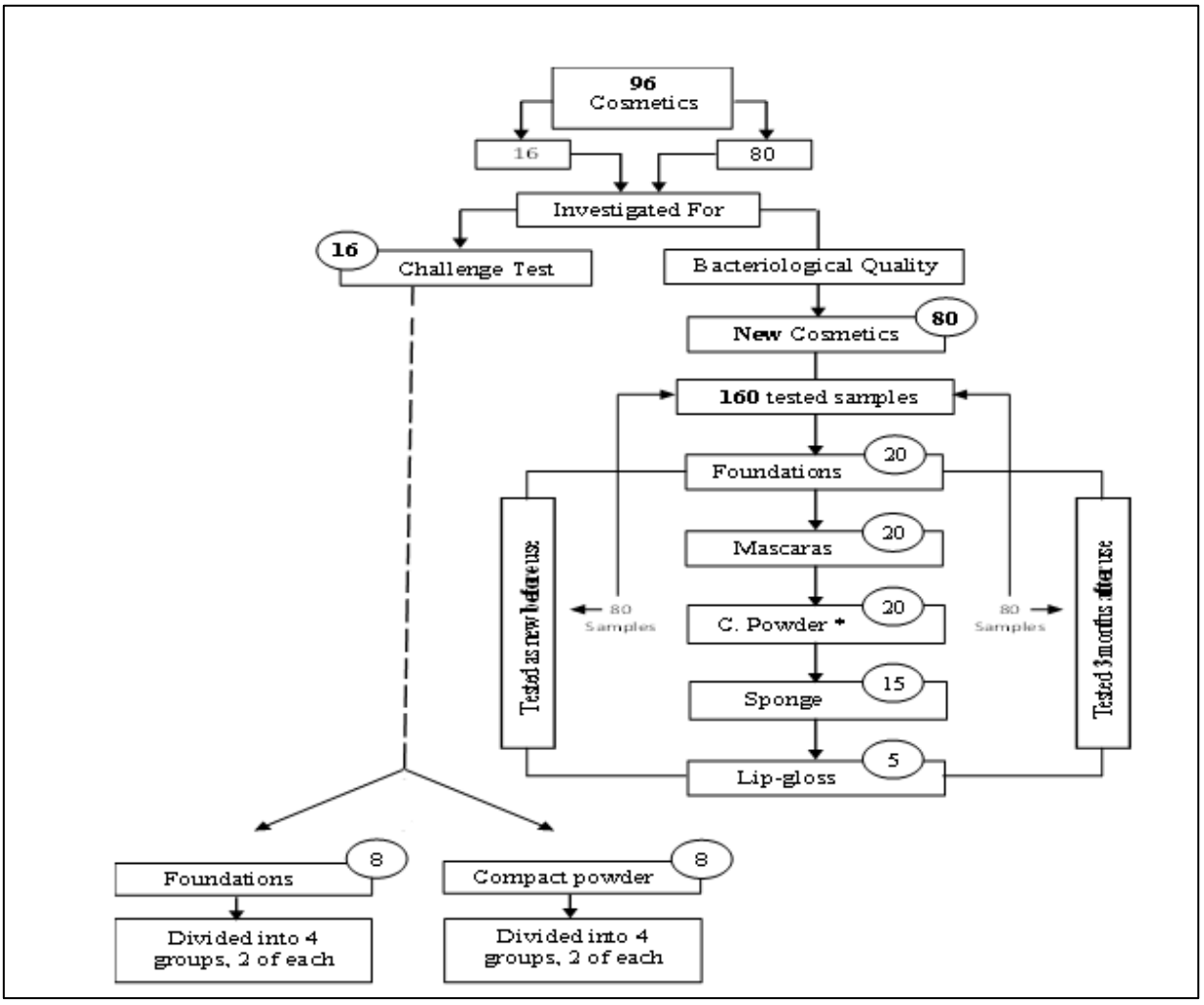

Figure 1: Schematic representation of investigation plan showing numbers and distribution of cosmetic's samples.

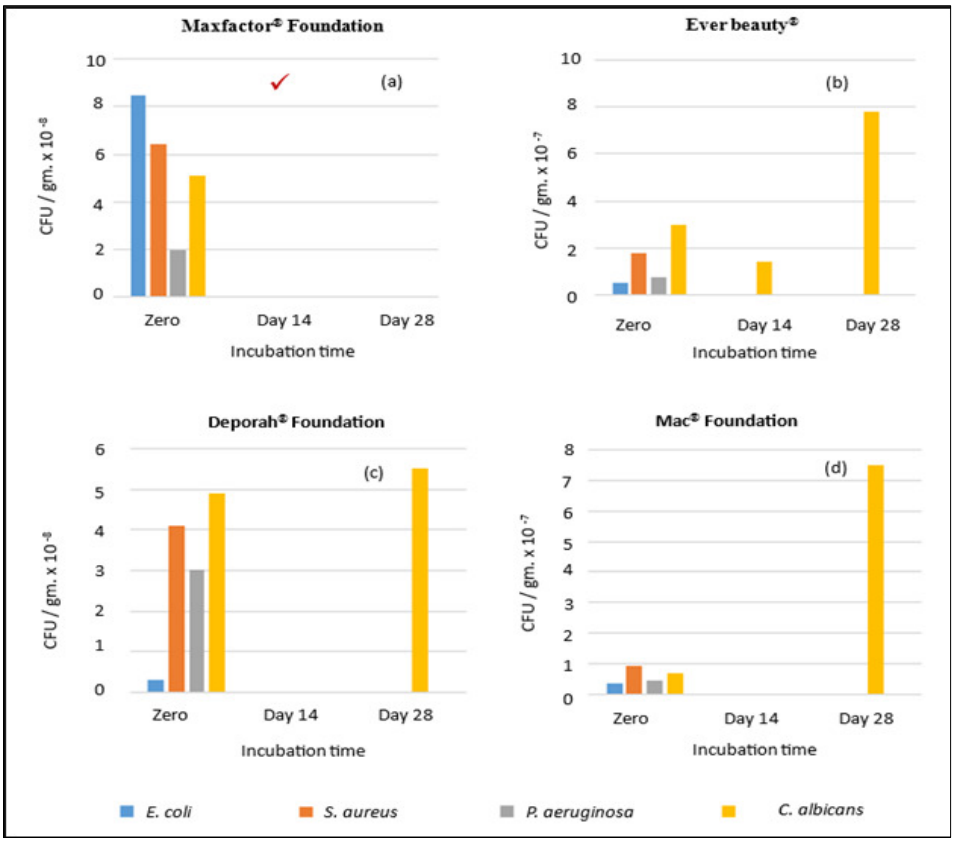

Figure 2: Viable counts of Foundation cosmetic products

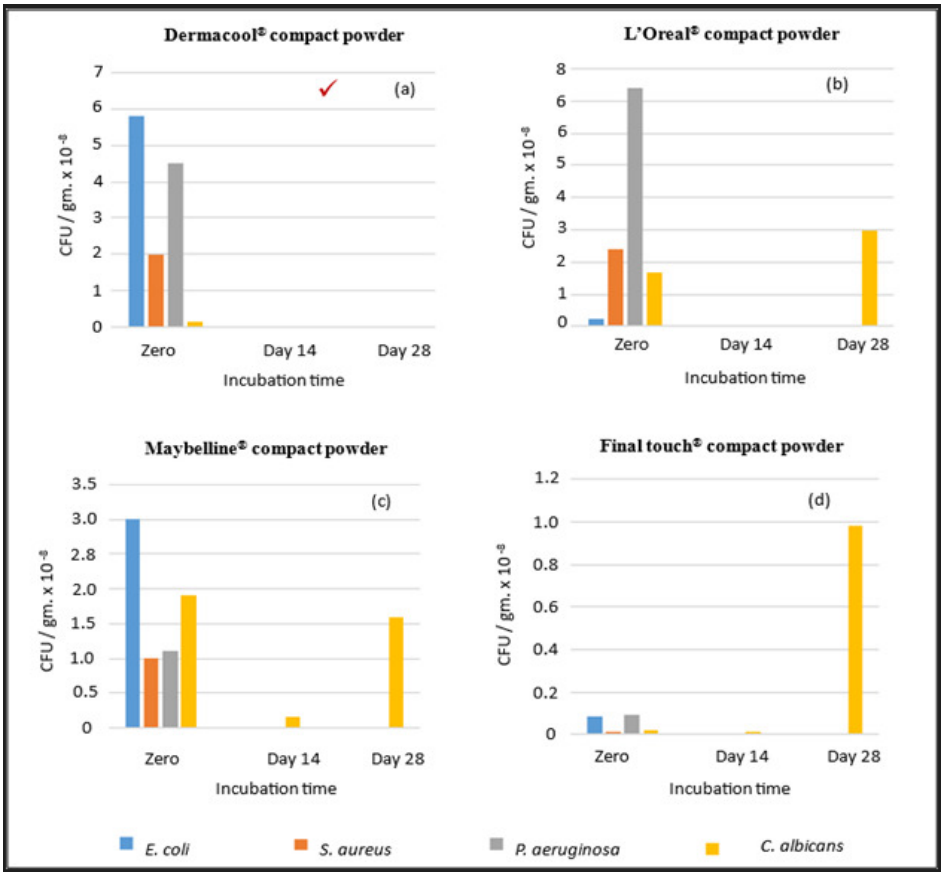

Figure 3: Viable counts of compact powder cosmetic products

DISCUSSION

Eighty (80) new (unopened) cosmetic products of different trademarks (table 1) were investigated for possibility of their microbial contamination. No microbial contamination was detected in 79 of tested products of foundation, compact powder, sponge and lip-gloss except for one mascara sample of *Maybelline*® (1/20) was contaminated with *P. aeruginosa.* Same result was obtained from study carried out on 2005 in which no microbial contamination was detected in 91 examined cosmetic samples (Campana *et al*., 2006). One case of death was reported by immune compressive patient after using shampoo contaminated with *P. aeruginosa*. (Birteksoz tan *et al*., 2013). A research study carried out in 2009 revealed contamination of cosmetic eye products with *S. aureus, S. epidermidis* and *S. warneri*. Other study by Muhammed H. j. concluded that mascara samples were more contaminated than the other tested products and the contaminated organisms were *S. epidermidis, K. pneumonia, P. aeruginosa* and *C. albicans.* (Muhammed *et al*., 2011). Moreover, eye infections were noted after using cosmetic products contaminated by *P. aeruginosa* (Budecka and Kunicka-Styczyńska, 2014). The likely source of these organisms is from the hands of users, as moisturizers are often used after or independent of washing. (Campana *et al.,* 2006). The detection of varying types of microorganisms in the used cosmetic products was caused mainly by the volunteers whilst using the cosmetics and by contrast, no microbial contamination was found in new (un-used) packaged products. In the current study, *S. aureus* was detected in all Foundation products (2 *Maybelline*® samples, 1 *New well*® sample, and 1 *Mac*® sample) except for *Maxfactor*®, 2 lip-gloss samples of *Forever*® and *Deborah*® and 1 mascara sample of *New well*®). Other sample of foundation of *New well*® was contaminated with *Rhizopus* spp.; also *Rhizopus* spp. was detected in *Maxfactor*® Mascara; *P. aeruginosa* in *Maybelline*®Mascara. *K. pneumonia* a known respiratory pathogen was also observed in *Maybelline*®Mascara and Sponge. The present study is in agreement with the previous findings of (Ibegbulam-Njoku *et al*., 2016) who reported that, cosmetic products highly contaminated with bacteria and fungi including hazardous type such as: *S. aureus, P. aeruginosa and C. albicans. S. aureus* as the most commensal organism found in the skin was identified in Lip-gloss, Foundation and Mascara tested in this study. The current results agree with those obtained by many investigators (Baird, 1984; Behravan *et. al.,* 2005; Lundov *et. al.,* 2009; Elmorsy and Hafez, 2016) who found *S. aureus* and *P. aeruginosa* are the common pathogenic bacteria in cosmetic samples. All microorganisms recovered from cosmetic products tested in this study are opportunistic pathogens capable of finding easy access to sensitive area in human body such as eyes, nasal and oral cavity which may pose a health risk to the consumers causing various illness like blood stream, urinary infections and fungal peritonitis especially in immunocompromised patients (Trofa *et al*., 2008; Mahlen, 2011; De Bentzmann and Plesiat, 2011). the efficacy of different preservatives included in unopened (new) cosmetic products as shown in (table 2) was tested before use of cosmetics to evaluate the best preservative that can suppress and prevent the growth of bacteria and fungi. Results obtained from this study was evaluated according to the USP in a criteria with no less than second logarithmic reduction from the initial count and no more from the 14 and 28 days count for bacteria and no more from the initial calculated count at 14 and 28 days for yeast and molds (USP, 2003; Birteksoz tan *et al*., 2013). In accordance to the later acceptance criteria, four samples of two tested cosmetics were accepted namely; two of *Maxfactor*® foundations and two of *Dermacool*® compact powder samples, in which their preservatives were able to inhibit the growth and kill all inoculated standard of both bacteria and fungi at 14 and 28 days (table 5; figures 2a & 3a). While the other 12 samples were able to inhibit the growth and kill the inoculated standard bacteria at 14 and 28 days but unable to suppress or inhibit the growth of *C. albicans* suggested that the antimicrobial action of preservatives included in their formulations was not effective. Research studies by (Abdelaziz *et al*., 1989; Hugbo *et al*., 2003; Omorodion *et al.,* 2014; Gamal *et al.,* 2015) found cosmetic creams harbor high numbers of bacteria and fungi. Fungal contamination of some cosmetic products especially in creams can be attributed to the type of cream preparations which are often water in oil emulsions, with high concentrations of solutes and lowered water activity. Finally, the results presented in this study revealed presence of varying types of microorganisms in the used cosmetic products indicating contamination caused by the volunteers whilst using these cosmetics and no microbial contamination was found in approximately new (un-used) packaged products. This result comes in agreement with EU guidelines which stated that, in many cases organisms present in used cosmetic products but were prohibited in packaged (new) products (Scientific committee and consumer safety, 2016). Research studies have revealed that, significant threat of infection comes when consumers using cosmetic products contaminated with potentially pathogenic organisms such *E. coli, S. aureus, P. aeruginosa, C. freundii* and *Candida* species, practically when they applied around the mouth or eyes (Pascher, 1982; Dadashi and Reza, 2016; Eldesoukey *et al.,* 2016).

CONCLUSION

The recovery of pathogenic microbes in tested cosmetic products is clear indication of poor cleaning and hygiene during their use, also the infectivity of their preservatives led us to presume that, the challenge test should be performed not only during the preparation of preservative system in new cosmetic products, but also be applied during their use in order to evaluate the protection efficacy of these preservatives, as recommended by the European Directive 2003/15/CE.

All potential sources of contamination must be identified and monitored. In order to do so, four steps must be considered (1) inspection and control of raw materials; (2) inspection and control of manufacturing process; (3) inspection and control of final product delivery and finally; (4) monitoring the use of cosmetics by the consumers, especially ladies.

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