The Egyptian Journal of Hospital Medicine (Jul. 2016) Vol. 64, Page 258- 266

Evaluation of Preservative Capacity for Some Selected Cosmetic Products Found in the Market

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ABSTRACT

Aim of the study: To determine the preservative capacity of different cosmetic preparations commonly found in the Egyptian markets.

Methods: Microbiological evaluation of 74 cosmetic sample and preservative capacity test for cosmetic samples showing no microbial contamination using rejecting microorganisms by cup plate technique.

Results: It was found that 29 samples were contaminated at levels $>10^3$ or >500 (for baby care products) or contaminated with rejecting microorganisms or both. The preservative capacity was variable between the different types and brands of the tested cosmetics against bacteria ($P<0.05$), while for Candida albicans all samples of the different cosmetic types were of nearly the same effect ($p>0.05$).

Conclusion: The detection of microbial counts greater than the microbial limits standards and isolation of rejecting microorganisms are clear evidences of non-adherence to good Manufacturing Practices. Variable preservative capacity in some cosmetics may indicate its ability to withstand microbial contamination which leads to spoilage of these cosmetics.

Keywords: cosmetics, contamination, rejecting microorganisms, preservative capacity.

INTRODUCTION

The microbial contamination of cosmetic products is of concern worldwide due to possible negative consequences on the health of users and on product integrity[1]. Cosmetic industries are not obliged to produce sterile cosmetics. Nevertheless, they are liable to assure safety of the product to the potential consumer. Their microbiological load is strictly controlled at various manufacture stages and during shelf-life[2].

Contaminating microorganisms in cosmetics may cause spoilage of the product and when rejecting microorganisms are present, they represent a serious health risk for consumers worldwide[3]. Most cosmetics contain a lot of ingredients that are good for microbial growth also the production of cosmetics is not a sterile process, and at least the storage temperature is nearly optimal for microbial growth[2].

Preservatives are intended to be added to prevent microbial spoilage during production, to prevent contamination by consumers while in use, to kill low levels of contamination introduced during storage and repeated use, and hence prolong the shelf life of products and protect consumer from potential infections[1,4].

Preservatives are not used to mask contaminated raw materials and should not be used to treat contaminated products[5,6].

All Preservatives incorporated into cosmetics have limitations on some microorganisms they are active against, regarding the physical characteristics of the products and also the manufacturing processes utilized during production of the finished product. So, it is critical to ensure that the preservatives selected for a particular product are matched to the physical and chemical requirements of the product and will provide protection against the full spectrum of microorganisms likely to be encountered[5].

MATERIALS & METHODS

Samples:-

Seventy four commercially available cosmetic products samples, from sixteen different brands were purchased from the Egyptian market and employed in this study, and they are represented in **Table 1.** The samples were analyzed as soon as possible upon their arrival.

Microorganisms:-

The rejecting microorganisms that were isolated in the present study were *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Candida albicans*. Some of these isolates were reused in

Received: 11/07/2016
Accepted: 17/07/2016
DOI : 10.12816/0029018

the preservative capacity test. Bacteria were kept on tryptic soya agar slants and *C. albicans* was kept on Sabouraud's dextrose agar slant. All slants were kept at 4 C^o.

Total bacterial and fungal counts:-

The collected samples of cosmetic products were subjected for determination of the total bacterial and fungal counts. One ml of each sample was aseptically measured and serially diluted in the neutralizing solution[7]. For bacterial growth, 0.1 ml was spread on tryptic soya agar (Oxoid) and incubated at 37^{O}C for 24-48hr. While for growth of fungi, 0.1 ml was spread on Sabouraud's dextrose agar (Oxoid) and incubated at 28+2^{O}C for 5-7days. The microbial counts was determined as colony-forming unit per I ml of sample (cfu/ml). Each sample was assayed in triplicate plates and the average values were calculated[8].

Identification of microbial contaminants:-

Pure cultures of isolated bacteria were preliminarily identified by Gram stain and colonial morphology of growth characteristics on selective, non-selective and differential culture media[7,8]. Further identification was carried by **MicroScan ® MIC/Combo Panels (***Siemens Healthcare Diagnostics Inc., UK).* Corn Meal Agar (Oxoid) and light microscope were used for the detection of chlamydospore formation of Candida species[9].

Determination of Preservative Capacity by cup-plate technique:

The cosmetic samples that were found to be free from microbial contamination were subjected to preservative capacity testing against the selected *Staphylococcus aureus, P. aeruginosa, E. coli, K. pneumoniae, E. cloacae and C. albicans*. The preservative capacity test was done on Mueller Hinton agar and Sabaraud's dextrose agar plates for bacteria and *Candida albicans*, respectively. Aliquots of 0.1mL containing $1x10^6$ cfu/mL of each microorganism were separately inoculated onto the agar plates and left for 15 minutes before being cup plated with 0.4 mL of each of the hair gel and hair cream samples. While for wet wipes, 6 mm disc of each sample was placed on the inoculated agar plate surface. Observation and determination of the zones of inhibition (ZI) were preceded with an aerobic overnight incubation at 37C^o for bacteria and *C. albicans.* After incubation, zones of inhibition (ZI) were measured in millimeter. Each Sample was tested in triplicate and the average values for the zones of inhibition were calculated[10, 11].

Statistical methods:-

The data were analyzed by Statistical Package for the Social Sciences (SPSS) for Windows version 15.0. Student t-test and ANOVA-test (F-test) were used for comparison between types for data presented by mean ± SD. Level of significance: P-value>0.05 is not statistically significant. P-value<0.05 is statistically significant.

RESULTS

Forty three samples out of 74 examined cosmetic samples were found to be generally contaminated with microorganisms, but 29 samples out of the 43 contaminated samples were contaminated either to levels $>10^3$ or >500 (for baby care products) or contaminated with rejecting microorganisms or both. Results reveal that out of the 25 hair gel samples representing 5 brands, 13 samples were contaminated with bacteria and 2 samples were found to be contaminated with fungi. Out of 25 hair cream samples representing 5 brands, 18 samples were contaminated with bacteria and no samples were contaminated with fungi. While, out of 24 wet wipes samples representing 8 brands, 12 samples were contaminated with bacteria, and 2 samples were contaminated with fungi. The incidences and level of microbial contamination are present in **Table 2**.

Out of the 29 contaminated samples, nine samples were found to be contaminated with rejecting microorganisms. Four isolates of *S. aureus* were isolated from two hair gel samples and two wet wipes samples. Three isolates of *P. aeruginosa* were isolated from one hair gel sample and two hair cream samples. Three isolates of *E. coli* were isolated from two hair cream samples and one wet wipes sample. Two isolates of *K. pneumoniae* were isolated from one hair cream sample and one wet wipes sample. One isolate of *E. cloacae* was isolated from one hair gel sample, while three isolates of *C. albicans* were isolated from two hair gel samples and one wet wipes sample, some samples were contaminated with more than one rejecting microorganisms.

The 31 uncontaminated samples were tested for their preservative capacity against some isolates from the pre-isolated rejecting microorganisms as illustrated in **Tables 3, 4 and 5.**

Table 3, show that the hair gel samples (No. 1, 2, 3, 4, and 5, of brand G-A) were capable of inhibiting most of the testing microorganisms, giving zones of inhibition ranging from 10mm to 40mm. They gave high effect against *K. pneumoniae* as the zone of inhibition range from 29mm to 40mm. But the samples (No. 6, 7, 8, 9 and 10 of brand G-B), had weak effect on the microorganisms giving zones of inhibition ranging from 7.5mm to 16mm. While, sample No. 9 had no effect against *S. aureus, P. aeruginosa* and *E. coli* and sample No. 10 had no effect against *E. coli* and *E. cloacae*. The samples (No. 24 and 25 of brand G-E), were not able to inhibit any of the bacteria but they inhibited *C albicans* weakly giving zones of inhibition of 11.5 mm and 9 mm.

As illustrated in **Table 4.** the hair cream samples (No. 42 and 45 of brand C-G) had weak inhibitory effect against, *K. pneumoniae,* and *E. cloacae,* giving zones of inhibition of 10mm and 14.5mm, while they had no effect toward the other microorganisms. For the samples (No. 46, 47, 48, 49 and 50 of brand C-P), the results show that there were different degrees of inhibition against all the testing microorganisms giving zones of inhibition ranging from 8mm to 15mm.

The wet wipes samples capacity showed in **Table 5.** revealed that the samples (No. 53 of brand W-H and No. 62 of brand W-K) were effective against *K. pneumoniae* and *E. cloacae* giving weak inhibitory effect of 10mm and 12mm and they were not effective against the other testing microorganisms. But, the sample (No. 65 of brand W-L) was able to inhibit all the microorganisms giving varying zones of inhibition ranging from 10mm to 16mm.

The samples (No. 66, 67 and 68 of brand W-M) were not effective against *P. aeruginosa* and *E. cloacae,* but were effective against the other microorganisms giving varying zones of inhibition ranging from 10mm to 20mm**.** The samples (No. 69, 70 and 71 of brand W-N) were able to inhibit all the microorganisms giving zones of inhibition ranging from 12mm to 20mm. Also the samples (No. 72, 73 and 74 of brand W-O) were effective against most of the microorganisms giving zones of inhibition ranging from 10mm to 15mm, but they were not effective against *P. aeruginosa*.

The statistical analysis comparison between the preservative capacities of the different types of cosmetics against the testing rejecting microorganisms is illustrated in **Table 6.** The data showed that the preservative capacity differed between the different tested cosmetic products and the different microorganisms.

DISCUSSION

Hair gel, hair cream and wet wipe products, are cosmetic products that have great role in recovery and maintains of hair and skin health. Batch numbers differentiate and regulate the quality control and recalling processes, this makes the importance of batch number for consumers as well as manufacturers[12].

In this study, ten brands were found to be contaminated with microorganisms. Three of them (G,I and L) have no batch number and the other seven (C, D, E, F, J, H and K) have no batch number which make their recalling so difficult.

In the present study, the results revealed that 58% of examined samples were contaminated with bacteria and 5% were contaminated with fungi at different levels. for hair gel type samples, 10 samples were contaminated with bacteria at levels more than 10^3cfu/ml, for hair cream samples, 10 samples were contaminated with bacteria at levels more than 10^3cfu/ml, For wet wipes samples, 9 samples were contaminated with bacteria at levels more than 10^3cfu/ml, while the contamination with fungi at level more than 10^3 cfu/ml was found in one samples.

In other studies, Hugbo *et al*. found that 90% of examined cosmetic cream products were contaminated with bacteria at levels more than 10^2 and 10^3 cfu/ml and 70% were contaminated with moulds at less level than bacteria.[13] Behravan *et al.* evaluated 24 cosmetic samples and found that 67% of samples were contaminated with G-ve as well as G+ve bacteria at levels ranging from less than 10^2 to more than 10^3 cfu/ml[3].

Onurdag *et al.* investigated 73 cosmetic samples and found that 12% of examined cosmetic samples were contaminated at varying levels[4]. Mwambete and Simon found 70% of cosmetic products investigated yielded bacterial contaminants, while 40% yielded fungal contaminants at levels more than 10^3 cfu/ml[10]. El-Bazza *et al.* evaluated the microbial count in 50 cosmetic cream samples; he found that 46% of samples were contaminated, where 40% were contaminated with bacteria 38% of samples

were contaminated with fungi in the levels more than 10^3 cfu/ml[14].

The results of detection of rejecting microorganisms using selective and nonselective media showed that, four isolates of *S. aureus*, three isolates of *P. aeruginosa*, three isolates of *E. coli*, one isolate of *E. cloacae*, two isolates of *K. pneumoniae* and three isolates of *C. albicans* were isolated from 9 samples (12%) of evaluated cosmetic samples.

In consistence with the present study, Hugbo *et al.* isolated *S. aureus* from contaminated samples[13] and Behravan *et al.* isolated the same bacteria together with *E. coli* from contaminated samples[3], also Onurdag *et al.* isolated *Candida spp*., *S. aureus* and *E. coli*[4], as well as Mwambete and Simon and Tan *et al.* isolated *S. aureus, P. aeruginosa* and *E. coli* [10,15].

Sutton and Jimenez found in an analysis conducted on 642 microbiologically-related recalls over the years 2004-2011. The majority of the recalls came from personal care products due to contamination with rejecting microorganisms (*P. aeruginosa, E. cloacae, E. coli, S. aureus* and *Salmonella spp*.) as the most prevalent reason for recalls[16].

The microbial evaluation of cosmetic samples was done in presence of neutralizing solution for preservatives to avoid false negative results[8]. The microbial limits values of finished cosmetic products in the United States pharmacopeia and in the British pharmacopeia, are set according to the products category, which are; category 1 (eye area products and baby care products) and category 2 (non-eye area products). Microbial counts below 500 cfu/g or ml for category (1) and below 1000 cfu/g or ml for category (2) absence of rejecting microorganisms (*P. aeruginosa, S. aureus, and C. albicans*) are accepted for both categories[17,18]. Detmer *et al.* in the environmental project No. 1336 following to the Danish Ministry of the Environment added to the previous limits that it is generally acknowledged that neither the occurrence of *E. coli* nor other members of *Enterobacteriaceae* are acceptable in cosmetic products[19].

The presented data revealed that some cosmetic samples obeyed the accepted microbial limits and other samples did not obey it from the different examined cosmetic types.

The detection of microbial counts greater than the microbial limits standards and isolation of pathogenic microorganisms in finished products are clear evidences of non-adherence to good Manufacturing Practices guidelines, because microbial contaminants have been introduced into the products during manufacturing or packaging process[10, 20].

The zone of inhibition test is a good method for testing cosmetic products preservative capacity[10]. For testing the preservative efficacy, it is recommended to add strains isolated from the environment, water, or contaminated products. These strains live in the vicinity of or even inside the product, are well adapted to adverse conditions, and are often resistant to preservatives or even disinfectants[8,19]. So in the present study, from the isolated objectionable microorganisms, selected strains were used in the preservative capacity test.

The 31 samples free from microbial contamination representing three gel brands, two hair cream brands and six wet wipes brands were tested for their preservative capacity. The results showed that the preservative capacity of the different cosmetic types as well as brands was variable against each microorganism.

The preservative capacity between brand G-A and brand G-B against *S. aureus, P. aeruginosa, E.coli, K. pneumoniae,* and *E. cloacae* were statistically different and higher for brand G-A ($p<0.05$). On contrary, against *C. albicans*, when comparing the brand G-A, brand G-B and brand G-E, they nearly had the same capacity range and no statistical difference ($p>0.05$).

On comparing the preservative capacity between brand C-G and brand C-P, there was only observed capacity for brand C-P against *S. aureus, P. aeruginosa, E. coli* and *C. albicans*. On contrary, against *K. pneumoniae, and E. cloacae* when comparing the brand C-P and brand C-G, they had nearly the same capacity range and no statistical difference ($p>0.05$).

When comparing the preservative capacity between brands W-L, W-M, W-N and W-O against *S. aureus and E. coli,* the statistical difference was insignificant ($p>0.05$). On contrary, against *P. aeruginosa, K. pneumoniae, E. cloacae* and *C. albicans* when comparing the different brands, they were statistically different and the highest capacity was for brand W-N ($p<0.05$).

Some ingredients that are usually incorporated into cosmetics tend to reduce the efficiency of preservatives or presence of agents

that create a favorable environment for microbial growth. Probably this may explain the observed variability of the cosmetic preservative capacity[10].

In this study, when statistically comparing the three different types of personal care products, it was observed that hair gel, hair cream and wet wipes have nearly the same preservatives capacity range against *S. aureus* ($p>0.05$). But against *K. pneumoniae* and *E. cloacae*, the highest capacity was for hair gel type ($p<0.05$). While, against *P. aeurginosa* and *C. albicans*, wet wipes type had the highest preservative capacity range ($p<0.05$). on the other hand, against *E. coli*, the wet wipes have the highest preservative capacity ($p<0.05$). So, the preservative capacity of the tested cosmetic products differs between the different testing microorganisms.

In consistence with the present study, Mwambete and Simon examined ten samples each representing a brand of different cosmetic type for their preservative capacity. They found that preservatives capacity was variable, where the ten brands showed good preservation capacity against *S. aureus, P. aeruginosa, Escherichia coli and C. albicans*. Five cosmetic samples were capable of inhibiting growth of all the testing microorganisms while the other 5 cosmetic samples proved to be ineffective against *C. albicans*. One cosmetic sample, exhibited potent antimicrobial activity against *E. coli*, and the samples were observed to be of equal efficacy[10].

On the other hand, David *et al.* found that preservatives capacity was variable, where they examined 10 samples each representing a brand of wet wipes for their antibacterial capacity, the study showed that in all the brands of wipes examined, and one sample was the most potent in inhibiting all the testing bacteria. One other sample was most effective against *S. aureus* with the widest zone. Two samples inhibited all the test bacteria except *E.coli* and *P. aeruginosa*. Other four samples were each effective against three of the test bacteria. In contrast, all the organisms grew in the presence of three samples and were therefore considered resistant to the products indicating lack of activity of the wipes preservation[11].

The low antimicrobial capacity of some cosmetics and difference in capacity between the different types of personal care products can be due to the interaction with the product's ingredients, partition of the active antimicrobial agents into insoluble phases of the cosmetic. Previous research has shown that creams, which are widely used in cosmetics, are occasionally prone to microbial contamination as a result of the preservatives partitioning into oily phase of the cream, while contaminants flourish in the aqueous phase now deprived of preservatives[10,21].

CONCLUSION:

The real problem was not only the heavy contamination on the examined samples but also, the contamination with pathogens, which played a great role in spoilage of cosmetics and changing its nature. The preservation capacity was variable between the different brands as well as between the different cosmetic types against bacteria. For *C. albicans* all cosmetic samples as well as the cosmetic types were of nearly the same effect, although some brands did not have any capacity against bacteria and /or *C. albicans*. Restricted control is recommended to reduce the marketing of microbial contaminated and rued preserved cosmetic products which may have series health risks to consumers.

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Table 1: cosmetic samples used in the present study

No. of cosmetic Samples	Types of cosmetic	Cosmetic Brands	Baby Care Brands	Brands with Batch No.	Expire date
25	Hair Gel (G)	A, B, C, D & E	-	A,B	+
25	Hair Cream (C)	C, F, D, G & P	-	G	+
24	Wet Wipes (W)	H, I, J, K, L, M, N & O	J, M & N	I, L, M & N	+
No= number, + = present on label					

Table 2: Level of microbial contamination of the tested cosmetic samples.

Type	Brand code	No. of Examined S.	No. of Contaminated S.	No. of contaminated S. with Bacteria		No. of contaminated S. with Fungi		Presence of rejecting M.O.
				$<10^3$ or <500*	$>10^3$ or >500*	$<10^3$ or <500*	$>10^3$ or >500*	
Hair Gel	G-A	5	0	-	-	-	-	-
	G-B	5	0	-	-	-	-	-
	G-C	5	5	2	3	1	-	+
	G-D	5	5	1	4	1	-	+
	G-E	5	3	-	3	-	-	-
Total	5	25	13	3	10	2	-	-
Hair Cream	C-C	5	5	3	2	-	-	+
	C-F	5	5	2	3	-	-	-
	C-D	5	5	-	5	-	-	+
	C-G	5	3	3	-	-	-	-
	C-P	5	0	-	-	-	-	-
Total	5	25	18	8	10	-	-	-
Wet wipes	W-H	3	2	1	1	1	-	-
	W-I	3	3	-	3	-	-	-
	W-J **	3	3	-	3	-	1	+
	W-K	3	2	-	2	-	-	-
	W-L	3	2	2	-	-	-	-
	W-M **	3	0	-	-	-	-	-
	W-N **	3	0	-	-	-	-	-
	W-O	3	0	-	-	-	-	-
Total	8	24	12	3	9	1	1	-
No.=Number, S.= Samples, M.O.= microorganisms, **=Baby care products, *= standard microbial limit for Baby care products.								

Table 3: Preservative capacity of the tested hair gel samples.

Brand code	Sample N0.	Inhibition zones (mm)					
		S. aureus	*P. aeruginosa*	*E. coli*	*K. pneumoniae*	*E. cloacae*	*C. albicans*
G-A	1	12.5	12	12.5	30	18	12
	2	12	10	11	31	16	12.5
	3	14.5	13	11.5	29	19	13
	4	16	12.5	12	30	19.5	12
	5	16	13	12	40	17	14
Mean ± SD		14.2 ± 1.8	12.1 ± 1.2	11.8 ± 0.57	32.0± 4.5	17.9 ± 1.4	12.7 ± 0.8
G-B	6	13	10	10	14	8	16
	7	9.5	8	9	11	10	7.5
	8	11	9	10	12	11	8.5
	9	NZI	NZI	NZI	14	12	9
	10	8.5	10	NZI	13	NZI	15
Mean ± SD		10.5 ± 1.9	9.3 ± 0.95	9.66 ± 0.57	12.8 ± 1.3	10.3 ± 1.7	11.2 ± 3.97
G-E	24	NZI	NZI	NZI	NZI	NZI	11.5
	25	NZI	NZI	NZI	NZI	NZI	9
Mean ± SD		----------	----------	-------	----------	----------	10.25 ± 1.76
Sig. test & P-value brands		t- test = 2.8 P= 0.02*	t- test = 3.75 P= 0.007*	t- test = 5.1 P = 0.002*	t- test = 9.1 P= 0.000*	t- test = 7.3 P = 0.000*	F-test = 0.67 P= 0.53
G=hair gel, NZI=no zone of inhibition, F-test=(ANOVA test) between the 3 brands, t-test=(student t-test) between 2 brands, * P-value < 0.05 is considered significant.							

Table 4: Preservative capacity of the tested hair cream samples.

Brand code	Sample N0.	Inhibition zones (mm)					
		S. aureus	*P. aeruginosa*	*E. coli*	*K. pneumoniae*	*E. cloacae*	*C. albicans*
C-G	42	NZI	NZI	NZI	14.5	10	NZI
	45	NZI	NZI	NZI	12.5	11	NZI
Mean ± SD		-------	-------	----	13.5 ± 1.4	12.2 ± 1.3	---
C-P	46	12	10	9	13	10	12.5
	47	11	11	10	11	8	11.5
	48	13	10	9	11	8	11.5
	49	10	10	8	12	9	13.5
	50	15	9	10	14	10	13
Mean ± SD		12.2 ± 1.9	10.0 ± 0.7	9.2 ± 0.8	12.2 ± 1.3	9.0 ± 1.0	12.4 ± 0.89
Sig. test & P-value between brands		------	-----	----	t-test = 2.4 P = 0.29	t-test = 3.5 P = 0.11	------
C=hair cream, NZI=no zone of inhibition. T-test=(student t-test) between 2 brands, P-value > 0.05 is considered not significant.							

Table 5: Preservative capacity of the tested wet wipes samples.

Brand code	Sample N0.	Inhibition zones (mm)					
		S. aureus	*P. aeruginosa*	*E. coli*	*K. pneumoniae*	*E. cloacae*	*C. albicans*
W-H	53	NZI	NZI	NZI	10	10	NZI
W-K	62	NZI	NZI	NZI	12	12	NZI
W-L	65	10	15	12	10	13	16
W-M	66	14	NZI	19	12	NZI	14
	67	15	NZI	11	20	NZI	13
	68	14	NZI	10	19	NZI	14
Mean ± SD		14.3± 0.57	-----	013.3± 4.9	17.0 ± 4.3	-----	13.6 ± 0.57
W-N	69	15	16	12	20	16	16
	70	15	19	13	18	15	17
	71	12	16	12	19	15	16
Mean ± SD		14.0± 1.7	17.0 ± 1.7	12.3± 0.57	19.0 ± 1.0	15.3 ± 0.57	16.3 ± 0.57
	72	10	NZI	11	12	12	13
	73	10	NZI	10	11	10	12
	74	15	NZI	12	10	10	13
Mean ± SD		11.6 ± 2.8	------	11.0 ± 1.0	11.0 ± 1.0	10.6 ± 1.15	12.6 ± 0.57
Sig. test & P-value between brands		F-test = 1.6 P = 0.2	-----	F-test= 0.48 P = 0.6	F-test= 7.4 P = 0.02*	t-test= 6.26 P= 0.003*	F-test= 32.3 P= 0.001*
(W) wet wipes, (NZI) no zone of inhibition. F-test (ANOVA test) between the 3 brands, t-test (student t-test) between 2 brands, *P-value < 0.05 is considered significant.							

Table 6: Statistical analysis showing the difference in preservative capacity between the different types of cosmetics.

Type of Microorganism	Hair gel		Hair cream		Wet wipes		Sig. test & p- value
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	
S. aureus	8.5-16	12.5 ± 2.65	10- 15	12.2 ± 1.9	10-15	13.0 ± 2.3	F-test= 0.2 P=0.8
P. aeruginosa	8 - 13	10.8 ± 1.8	9- 11	10.0 ± 0.70	15-19	16.5 ± 1.7	F-test= 22.2 P= 0.000*
E. coli	9- 12.5	11.0 ± 1.2	8- 10	9.2 ± 0.83	10- 19	12.2 ± 2.5	F-test= 4.14 P= 0.03*
K. pneumoniae	11-40	22.4 ± 10.5	11- 14.5	12.5 ± 1.36	10- 20	14.4 ± 4.3	F-test= 5.3 P= 0.01*
E. cloacae	8-19.5	14.5 ± 4.3	8- 11	9.4 ± 1.1	10- 16	12.6 ± 2.4	F-test= 5.6 P= 0.01*
C. albicans	7.5-16	11.6 ± 2.6	11.5- 13.5	12.4 ± 0.89	12- 17	14.4 ± 1.7	F-test= 4.6 P= 0.02*
F-test (ANOVA test) between the 3 types, * P-value < 0.05 is considered significant.							