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MICROBIAL QUALITY ASSESSMENT OF SOME BRANDS OF COSMETICS PRODUCTS SOLD IN INDIAN MARKETS

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ABSTRACT

Cosmetics are products created for skin & hair care for the intention of cleaning, beautifying and increasing the good-looking features. Almost all of the cosmetics used by humans precipitated a lot of destructive outcomes mainly because of the components delivered to the cosmetics as preservative, stabilizer, perfume and coloring agent. Present study was intended to identify and enumerate microorganisms spoiling usually used cosmetics samples. Beauty product must be free of pathogenic microorganisms and the entire aerobic count must be within the permitted values as prescribed by Bureau of Indian Standards. Total 33 brands of 10 categories of samples were studied. Almost all were found to be rigorously contaminated. The studied samples showed contamination with Gram positive bacteria such as Staphylococcus spp and Bacillus spp were specially detected. However, all tested samples were free from gram negative bacteria and moulds. The total viable bacteria were found to be within a range of 10^1-10^4cfu/g. Bacteria such as Bacillus spp & Staphylococcus spp were found in the range of 10^3-10^4cfu/g. Bacillus spp encountered in 84% of samples. From this study it was found that microorganisms such as Staphylococcus spp and Bacillus spp were found in almost all samples. Presence of those microorganisms might be responsible for ugly odour and spoilage of cosmetic products.

KEY WORDS

Quality Assessment, Cosmetics, Standard plate count, FDA, MLB.

INTRODUCTION

Cosmetics are product created for skin & hair care for the purpose of cleansing, beautifying and enhancing the attractive features [1]. Cosmetic means the articles intended to be rubbed, poured, or sprayed on, introduce into or otherwise applied to the human body or any part thereof for cleansing, beautifying, promoting attractiveness or altering the appearance and article intended for use as a component of any such articles; except that such term shall not include soap. Although cosmetics products have undergone many changes in modern times, the basic concept of using cosmetics to enhance the feature of good health has not changed. While cosmetics can be fun to use, the work that goes behind the scenes for creating them involves highly advanced science. ISO & FDA has issued guidelines for safety product and scheduled microbiological analysis should be done to reach the safety level [2]. The level of contamination in cosmetic products with aerobic bacteria should not exceed the US pharmacopoeia or FDA limit (non-eye area <1000cfu/g) and if the limit exceeds, serious skin problem to the user may be encountered [13]. Cosmetics often use vibrant colors that are derived from a while variety of sources, ranging from crushed insects to rust [10]. The realization of the dangers of many common ingredients also greatly affected the growing industry. Carmine was once the only bright red color approved by the FDA for use around the eye [12]. The strength of microorganisms depends on product composition, preservatives,

manufacturing hygiene, packaging, transport and storage. Water is essential for microbial growth and water-based products often have a limited durability, as they are sensitive to microbial growth. Raw materials can contribute to a significant level of microbial contamination to the finished product [3]. Testing of raw materials before use, especially those of natural origin is important. Purity after opening depends on the preservative ability of the product, suitability of the packaging, storage and application [8]. The following scenarios that can contribute to contamination of a cosmetic product, fingers are dipped in product, leakage of water into product, shampoo used by different people. Preservatives must be used at the lowest concentration that ensures their efficacy and this must be determined during the product development process. Some of these substances may also have allergic, irritating, and harmful effects on human health [4].Some commercial cosmetic products (lipsticks, lip glosses, eye shadows, and hair dye) in addition, in order to monitor the potential adverse effects of hair dye on hair quality, as well as the total body burden of heavy metals (Pb, Cd), a highly toxic metal[5].Kamal Kanta Das et al.,[6]performed the test for occurrence of microorganism in commonly used cosmetic samples to identify & enumerate microorganisms spoiling commonly used cosmetics samples. All samples were found to be rigorously contaminated with total viable bacteria within a range of 10^3-10^5cfu/g. proliferation of fungal spp was observed up to 10^3cfu/g.

MATERIALS AND METHODS

Collection of Sample

33 well-known brands (named mentioned later) of 10 categories of cosmetics (4 creams, 6 powders, 4 lotions, 3 face pack, 2 scrub, 3 toothpaste, 3 soap, 3 face wash, 2 eye liner, 3 mascara) with appropriate date of manufacturing and expiry were collected from different shops in market of Talod town during January 2017 to February 2017.

Preparation of Sample

Powders, creams, face wash, face pack, scrub, soap, tooth-paste & lotions were aseptically removed and weighed 1 g or ml sample in to 20×150 mm screw-cap test tube containing 1 ml sterile tween 80. Disperse products were dispersed in tween 80 with sterile spatula. 8 ml sterile MLB (Peptic digest of animal tissue 20g, Casein enzymichydrolysate 5g, beef extract 5g, yeast extract 2g, NaCl 5g, Sodium bisulphate 0.100g, Lecithin 0.700g, polysorbate 80 5g pH 7.0 ± 0.2) was added & mix thoroughly.

Eye liner and mascara were dispersed 10 g or ml of sample in to 90 ml of 0.1% peptone water. Add 8 ml sterile MLB and mix thoroughly.

Standard plate count

Samples were diluted decimally in MLB to obtain the appropriate dilution series 10^{-1} to 10^{-3}. Thoroughly mix dilutions and pippet 0.1 ml of each dilution on to surface of Nagar media (peptone 10g, Meat extract 3.0g, NaCl 5.0g, Agar 30g, D/w 1000ml, pH7.0- Himedia laboratories Pvt. Ltd.) in pre-labelled petri dishes. Spread inoculum over entire surface with bent glass rod that was first sterilised by dipping in 95% ethanol and quickly flamed to remove the ethanol. Use new spreader for each dilution. All the N-agar plates were maintained condition & incubated at 37°C for 24 to 48 hrs[9].

Count all colonies on plates containing number of colonies, and record results per dilution counted calculate and not down the results.

Identification by colony characteristics, morphological identification by gram's staining, spore staining, capsule staining, negative staining and final identification by biochemical tests.

Well isolated colony characteristics were observed for the identification like size, shape, margin, texture, elevation, opacity, consistency, pigmentation.

Selected bacterial strains were carried out to know gram's reaction, sporulating organism, capsulated organism as well as to check purity of culture suspension (Figure 1, 3, 5).

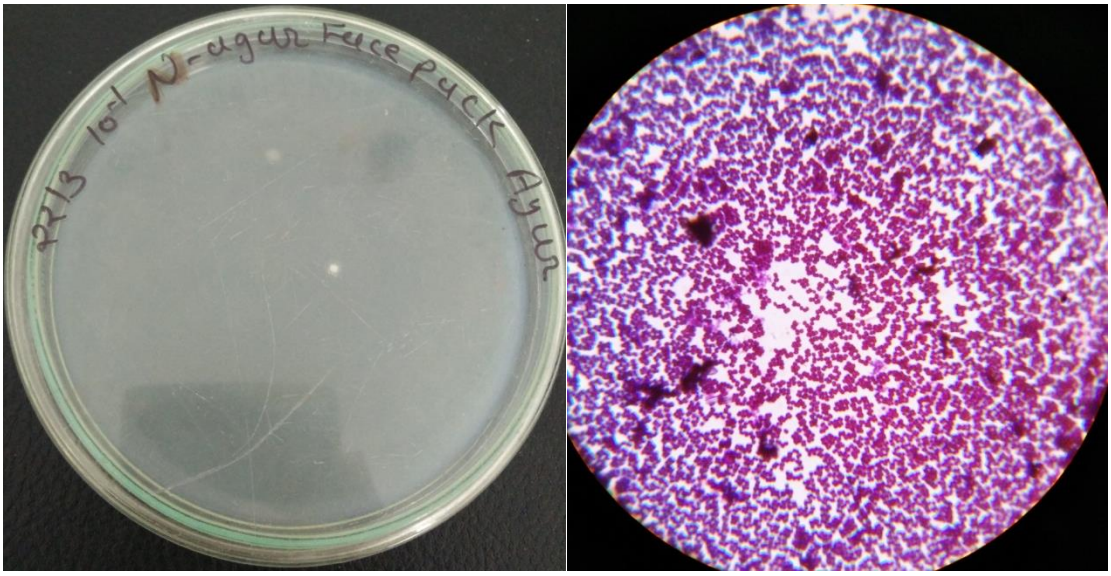

Figure 1: Gram's Staining of isolate no: 15 (Ayur face pack).

Figure 2: Various Biochemical tests of isolate no: 15 (Ayur face pack)

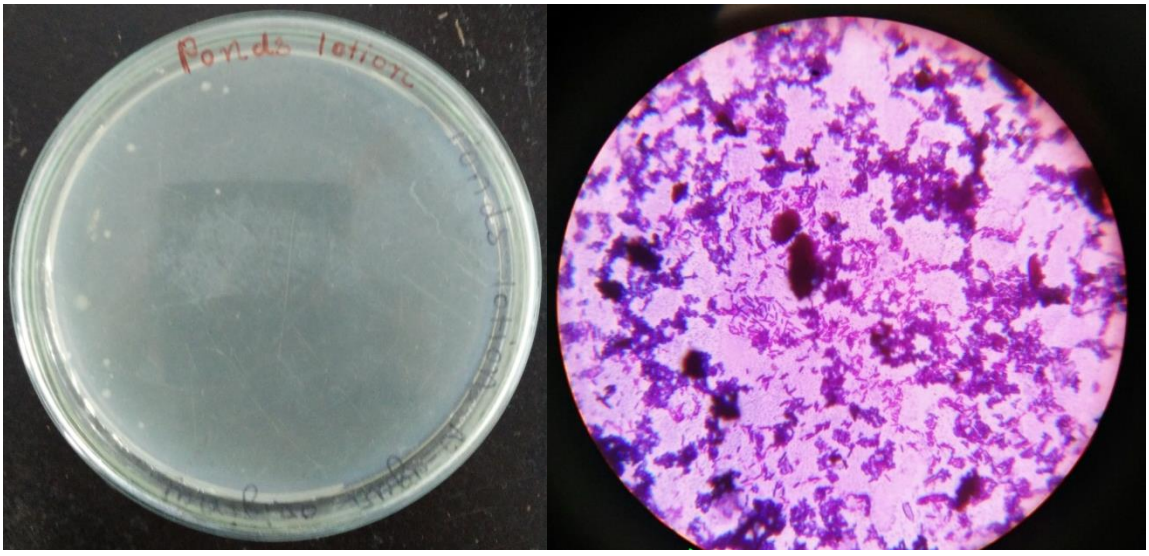

Figure 3: Gram's Stain of isolate no: 11 (Ponds lotion)

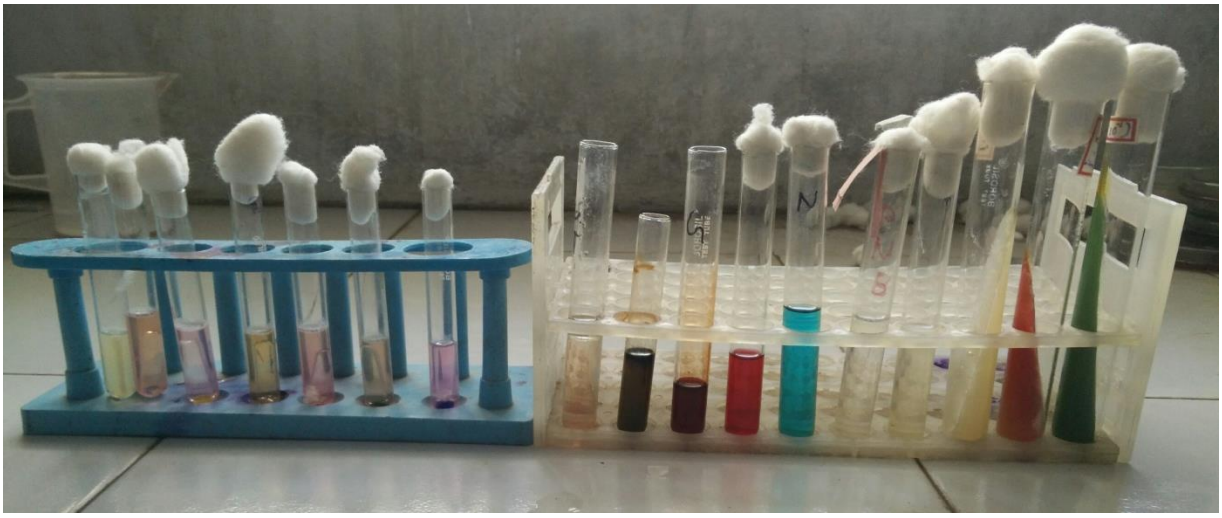

Figure 4: Various Biochemical tests of isolate no: 11 (Ponds Lotion)

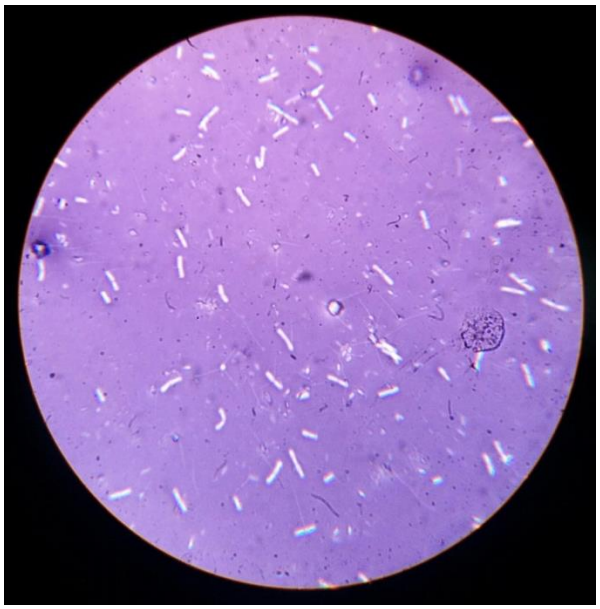

Figure 5: Negative Staining of isolate no: 11 (Ponds Lotion)

RESULTS AND DISCUSSION

After opening, cosmetics are not supposed to be sterile as they contain nutrients which support the growth of microorganisms. However, cosmetics products must have to be free from pathogens and total aerobic bacterial burden must be low which would not impair skin and mucous membrane. As the occurrence of skin disease is much more in developing countries due to the unhygienic environment, improper sanitation, and usage of microbiologically contaminated water. Therefore, the microbiological analysis of raw materials and final products of cosmetics is needed to obtain products with good microbiological qualities. Present study attempted to analyse various cosmetic products available in India for estimating the actual scenario. 10 categories of the cosmetic samples studied, all samples showed higher load of total viable bacteria up to 10^4cfu/g or ml & the isolates were biochemically identified (Table 1.1) (Figure 1, 2, 3). *Staphylococcus spp* were found in Ayur face pack, Himalaya &Everyuth scrub and Colgate, Anchor &Patanjali toothpaste. *Bacillus spp* were observed in (84%) 27 products in the range 10^4cfu/g. (Table 1.2).

Table: 1.1 shows the biochemical characteristics of all 33 isolates (products).

No	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
1	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	-
2	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	-
3	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	-
4	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	-
5	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	-
6	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	-
7	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	-
8	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	-
9	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	-
10	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	-
11	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	-
12	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	-
13	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	-
14	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	-
15	+	+	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	+	+
16	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	-
17	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	-
18	+	+	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	+	+
19	+	+	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	+	+
20	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	-
21	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	-
22	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	-
23	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	-
24	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	-
25	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	-
26	+	+	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	+	+
27	+	+	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	+	+
28	+	+	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	+	+
29	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	-
30	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	-
31	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	-
32	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	-
33	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	-

Where, A to Z: Name of biochemical tests,

No: Isolates (Cosmetics Products).

A. Glucose
B. Lactose
C. Xylose
D. Mannitol
E. Maltose
F. Sucrose
G. Indole production test
H. H_2S production test
I. Methyl red test
J. Voges-proskauer test
K. Citrate utilization test
L. Catalase test
M. Lipid hydrolysis test
N. Motility test
O. Ammonia production test

P. Starch hydrolysis test
Q. Dehydrogenase test
R. TSI test
S. Urea hydrolysis test

Table: 1.2 Aerobic microbial contents of cosmetic products

N0	Total count/g or ml	Isolated microorganism
1	2.0×10^{4}	*Bacillus spp*
2	3.0×10^{2}	*Bacillus spp*
3	1.5×10^{2}	*Bacillus spp*
4	2.2×10^{1}	*Bacillus spp*
5	1.2×10^{2}	*Bacillus spp*
6	1.2×10^{2}	*Bacillus spp*
7	1.0×10^{1}	*Bacillus spp*
8	8.0×10^{3}	*Bacillus spp*
9	1.0×10^{1}	*Bacillus spp*
10	5.0×10^{1}	*Bacillus spp*
11	4.1×10^{1}	*Bacillus spp*
12	1.0×10^{2}	*Bacillus spp*
13	5.0×10^{3}	*Bacillus spp*
14	1.5×10^{2}	*Bacillus spp*
15	3.0×10^{1}	*Staphylococcus spp*
16	1.0×10^{2}	*Bacillus spp*
17	3.0×10^{3}	*Bacillus spp*
18	1.1×10^{2}	*Staphylococcus spp*
19	1.0×10^{1}	*Staphylococcus spp*
20	1.0×10^{1}	*Bacillus spp*
21	2.0×10^{1}	*Bacillus spp*
22	2.4×10^{1}	*Bacillus spp*
23	2.8×10^{1}	*Bacillus spp*
24	1.0×10^{1}	*Bacillus spp*
25	1.1×10^{2}	*Bacillus spp*
26	4.0×10^{2}	*Staphylococcus spp*
27	3.0×10^{3}	*Staphylococcus spp*
28	3.0×10^{3}	*Staphylococcus spp*
29	4.3×10^{2}	*Bacillus spp*
30	5.3×10^{2}	*Bacillus spp*
31	2.9×10^{2}	*Bacillus spp*
32	3.0×10^{1}	*Bacillus spp*
33	7.0×10^{1}	*Bacillus spp*

Where, No: Isolates (Cosmetics Products).

1. Fair and lovely cream
2. Vicco cream
3. Clean & clear cream
4. Sun's cream lotus
5. Spinz powder
6. White tone powder
7. Pond's powder

8. Himalaya baby powder
9. Intasporin baby powder
10. Eva baby powder
11. Pond's lotion
12. Joy lotion
13. Santoor lotion
14. Nevia lotion
15. Ayur face pack
16. No marks face pack
17. Himalaya face pack
18. Everyuth scrub
19. Himalaya scrub
20. Santoor soap
21. Godrej no. 1 soap
22. Patanjali soap
23. Pond's facewash
24. Patanjali face wash
25. Angel face wash
26. Colgate toothpaste
27. Anchor toothpaste
28. Patanjali toothpaste
29. Clarion mascara
30. Easy paris mascara
31. Virgin Italy mascara
32. Clarion eye liner
33. Volume max eyeliner

The lowest bacterial count was observed in soap santoor within a range of 1×10^{1}cf/g, which was much lower than the previous study.Kamal Kanta Das et al., [6] reported the range between 2.1×10^{3} to 2.3×10^{5} cfu/g. The highest bacterial count was found in Himalaya baby powder in range of 8×10^{3}cfu/g was comparatively higher than earlier report of Micheal Macvren Dashen et al., [7] 1.3×10^{5} cfu/g in range. Medium bacterial count was found in ponds lotion in 4.1×10^{1}cfu/g compare with Kamal Kanta Das et al., [6] the total bacterial count was observed in range 3.5×10^{2}cfu/g to 1.9×10^{9}cfu/g, which was comparatively higher than present study. In the present study, the total viable count in eye products were in between 2.9×10^{2}cfu/g to 7×10^{1}cfu/g which was comparatively higher than the previous study (Tamalli M et al., 2013) of 1×10^{3}cfu/g.

As compared to previous studies of Kamal Kanta Das et al., [6], Tamalli et al.,[11], Micheal Macvren Dashen et al., [7].Almost all samples were found to be rigorously contaminated with bacterial load within a range of 10^{1}-10^{5}cfu/g or ml which is similar to present study. As specified earlier, raw materials, unhygienic handling and environmental condition may be responsible for contamination of products. Handling by the customers, application conditions and keeping conditions at that level would also impact the quality of the product. Some Chemicals such as lipid, Polysaccharide, Protein, Alcohol, Glucoside, storage temperature, product pH, Presence of $O_2$and poor activity of preservatives also can facilitate the growth of microbes. Presence of *Bacillus spp* might be responsible for unpleasant smell and spoilage cosmetics products.

CONCLUSION

From entire study it can be concluded that microbial contamination is a current issue&microbiological safety is one of the most critical parameterfor cosmetics quality. From this study it was found that microorganisms such as *Staphylococcus spp* was found present in all used cosmetics likeHimalaya scrub, Everyuth scrub and Ayurfacepack, Colgate, Anchor and Patanjali toothpaste whereas *Bacillus spp* was found in

almost all cosmetic products. The cosmetic industries have many compelling reasons to establish and maintain microbiological quality of its products. Factors include raw material quality, hygiene and training of manufacturing personal, establishment of sanitary design and materials. All of these factors are effective for the control of microbiological risk in cosmetic product.

In commercial establishments, where the product is used for customers, the direct contact of tube or applicator stick with numerous persons may result in cross contamination of skin inhabited flora and the rate of contamination could be increased.

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