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Microbial Analysis of Expired and 'In Used' Cream, Liquid and Matte Lipsticks

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Abstract

Easy access to blogs and social media has led to increased investing in cosmetics. Lipsticks act as mechanical vectors of microbes which spread by sharing, using testers in shops and malls, unhygienic applications of lipsticks on multiple costumers by salons etc. Emphasis on value for money by consumers leads to use of expired products which poses greater health risk. Mouth is the portal for entry of microbes directly into the gastrointestinal regions of the body which should be considered while using lipsticks. Samples of expired and in use lipsticks of different types were collected from volunteers in the study. Bacterial and fungal counts were determined using the spread plate method. Isolates were identified on the basis of colony characters, staining techniques and biochemical tests. Bacterial populations were isolated belonging to species of Gram positive bacteria viz. *Bacillus spp* (18.4%), *Streptococcus spp* (24.8%), *Staphylococcus spp* (41.4%) which included *S. saprophyticus*, *S. aureus* and *S. epidermidis* in all samples and Gram negative bacteria viz. *E.coli* (0.4%) and *Pseudomonas spp* (10%) were observed in expired and 'in used' liquid lipstick samples. Different Fungal populations were identified which belonged to *Aspergillus spp* (31.2%), *Penicillium spp* (19.8%), *Sacchromyces spp* (48.8%) and *Trichoderma spp* (0.2%). It was concluded that lipsticks often contain preservatives but some are still subject to microbial contamination. Total fungal count in expired lipsticks observed was lesser than the 'in used' lipsticks. Some of the bacterial isolates were found to be resistant to commonly known antibiotics.

Keywords: *Lipsticks, Contamination, Bacteria and Fungi.*

Introduction

Lipsticks are the most popular of all the cosmetic products. Presence of water and nutrients in cosmetics allow the growth of microorganisms. Furthermore, microorganisms also cause alterations such as offensive odors, changes in viscosity and color of lipsticks (Orus and Leranoz, 2005). Recently, cosmetics are extensively used for beauty by women. Beauty salons and sharing practices play an important role in possible transfer of skin infections due to the use of public make-up kits by the women. Cosmetic contamination has been frequently reported and has generated serious problems for consumers despite the improvements in the microbial standards of cosmetics. (Saeed and Asif, 2011) Lack of public awareness about quality, counterfeit products, production and expiration dates which are often not labeled on the cosmetic products during purchasing has been a serious problem in developing countries. Microbial growth is supported by high perspiration rate and bacteria such as *Bacillus* and *Staphylococcus aureus* are known to cause skin irritation. (Ezenna, 2017) Lipsticks consist minerals, growth factors, organic and inorganic compounds which are suitable for the growth of microorganisms. (Dadashi and Dehghanzadeh, 2016)

There have been innovations in formulations and textures of lipsticks in recent times. Matte lipsticks contain more filling agents like silica and cream lipsticks contain more waxes than oils. Sheer and longlasting lipsticks contain more oil, while long lasting lipsticks also contain silicone oil. There should not be more than 100 or 1000 colony-forming units per gram of the cosmetic, depending on the type of product according to a recommendation of the Scientific Committee on Consumer Safety of the European Commission. Pathogens like *Staphylococcus* or *E. coli* should not be detectable at all in the product. (Johnson, 1999)

The objective of this study is to compare the percentage of microbial contamination in expired and 'in used' cream, liquid and matte lipsticks. To find the type of lipsticks which have lower possibilities of microbial contamination when expired and during use and to test antibiotic resistance in bacterial isolates towards common antibiotics.

Materials and Methods

Total 120 samples of lipsticks were collected from volunteers out of which 60 samples were of expired cream, liquid and matte (20 each) and 60 samples were of 'in used' cream, liquid and matte lipsticks (20 each). All the media used were purchased from HiMedia Laboratories PVT LTD, India, for the isolation of bacteria and fungi like Nutrient agar (NA), MacConkey's agar, Mannitol salt agar (MSA), Eosin methylene blue (EMB) agar, Cetrimide agar and Rose Bengal Chloramphenicol (RBC) agar plates.

1gm/ml of each sample was introduced into 9 ml sterile saline solution. Ten-fold serial dilutions of the samples were made in the same diluent and 0.1 ml of 10^{-4} was plated out on the solid media mentioned above. The plates were incubated for 24 hours at 37°C for bacterial growth and 4 to 5 days at 25°C for fungal growth and the resultant colonies were counted. Total count of colony forming unit per gram or milliliter of lipstick samples were determined using the spread plate method. Bacterial isolates were identified on the basis of gram staining and biochemical tests while fungal isolates were identified by colonial characteristics and Lacto-phenol cotton blue staining using standard microbiological methods. Antibiotic susceptibility test was performed for isolating possible resistant bacterial strains in the sample using Kirby-Bauer Disk Diffusion Susceptibility Test Protocol and results were interpreted using CLSI standards.

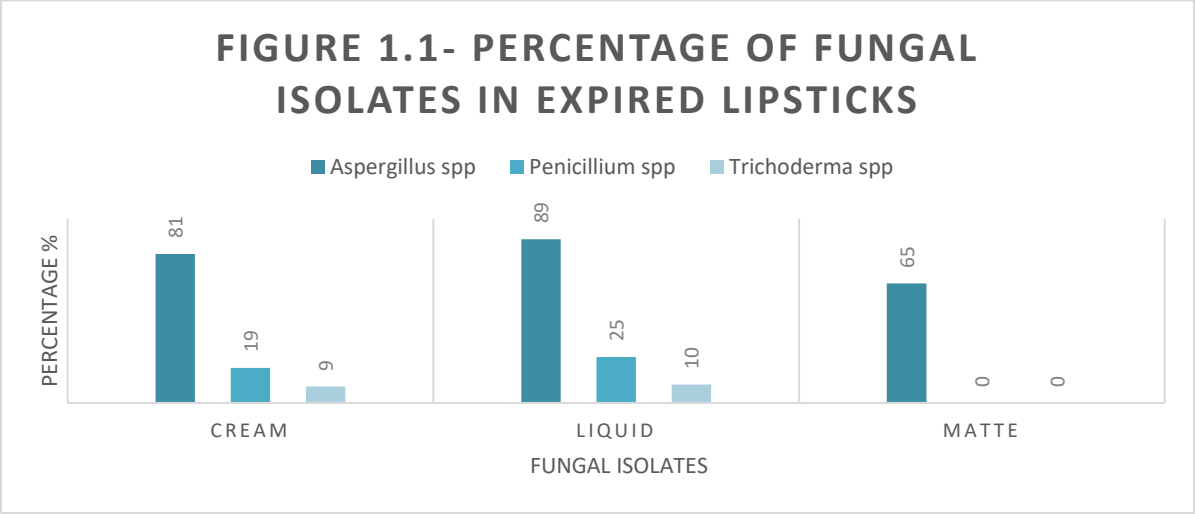
Results

Bacterial populations were isolated belonging to different species of Gram positive bacteria viz. *Bacillus spp* (18.4%), *Streptococcus spp* (24.8%), *Staphylococcus spp* (41.4%) which included *S. saprophyticus*, *S. aureus* and *S. epidermidis* in all samples and Gram negative bacteria viz. *E.coli* (0.4%) and *Pseudomonas spp* (10%) were observed in expired and 'in used' liquid lipstick samples. Total fungal count in expired lipsticks observed was lesser than the 'in used' lipsticks. Different Fungal populations were identified which belonged to *Aspergillus spp* (31.2%), *Penicillium spp* (19.8%), *Sacchromyces spp* (48.8%) and *Trichoderma spp* (0.2%). Some of the bacterial isolates were found to be resistant to commonly known antibiotics. Figure 1.1 and 1.2 represents percentage of bacterial and fungal isolates in expired lipsticks. Figure2.1 and 2.2 represents percentage of bacterial and fungal isolates in 'in used' lipsticks. The antibiotic susceptibility test was performed for all bacterial isolates (Table 1) out of which *E.coli* and *Pseudomonas spp* showed resistance to some antibiotics while other strains like *Staphylococcus spp*, *Bacillus spp* and *Streptococcus spp* were susceptible to the antibiotics used. *E.coli* showed resistance to Clindamycin, Oxacillin, Vancomycin and Azithromycin . *Pseudomonas spp* was observed to be resistant to Ampicillin, Choramphenicol, Penicillin G, Sulphatriad, Cephalothin, Clindamycin, Erythromycin, Oxacillin, Vancomycin and Linezoid.

Table 1- Zone of inhibition (in mm) showed by bacterial isolates in antibiotic disk diffusion susceptibility test

S.no	**Antibiotic**	**Conc (mcg)**	**Zone of inhibithion in mm**				
			Staphylococcus spp	***Bacillus spp***	***Streptococcus spp***	***Pseudomonas spp***	***E.coli***
1	Ampicillin	10	46	>55	NR	0	30
2	Chloramphenicol	25	30	>55	NR	0	30
3	Penicillin G	1units	50	>55	NR	0	10
4	Streptomycin	10	20	>55	NR	20	20
5	Sulphatriad	300	20	>55	NR	0	20
6	Tretracyclin	25	30	>55	NR	30	30
7	Cephalothin	30	30	>55	NR	0	20
8	Clindamycin	2	30	>55	NR	0	0
9	Erythromycin	15	40	>55	NR	0	0
10	Oxacillin	1	28	>55	NR	0	0
11	Vancomycin	30	20	>55	NR	0	0
12	Ciprofloxacin	5	30	>55	NR	40	40
13	Gentamicin	10	20	>55	NR	20	20
14	Linezolid	30	30	>55	NR	0	20

NR-No results under experimental conditions



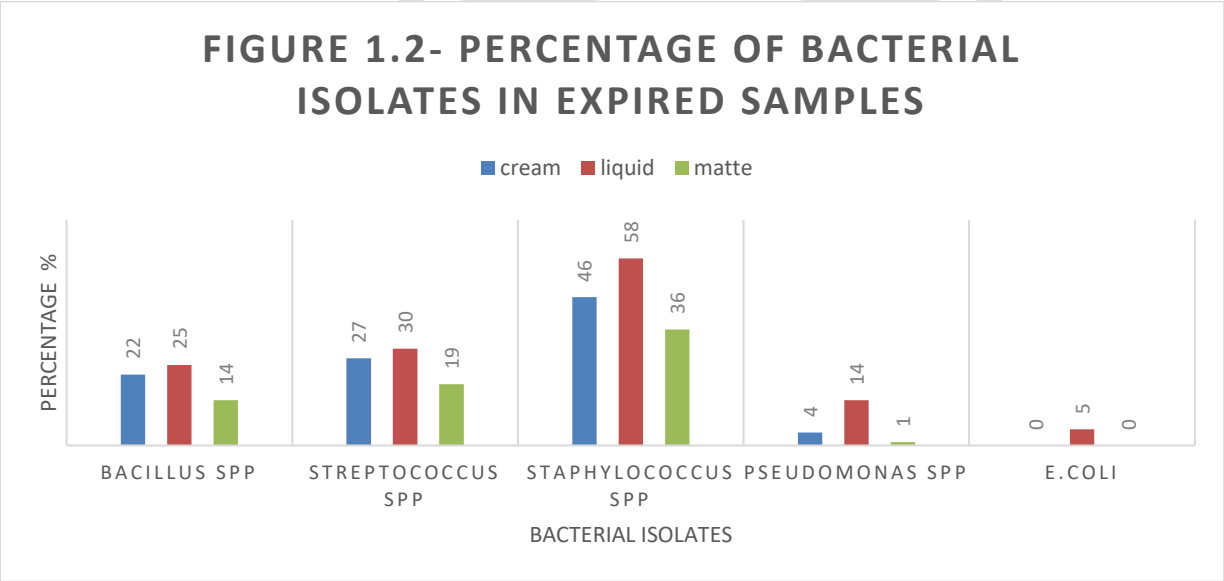
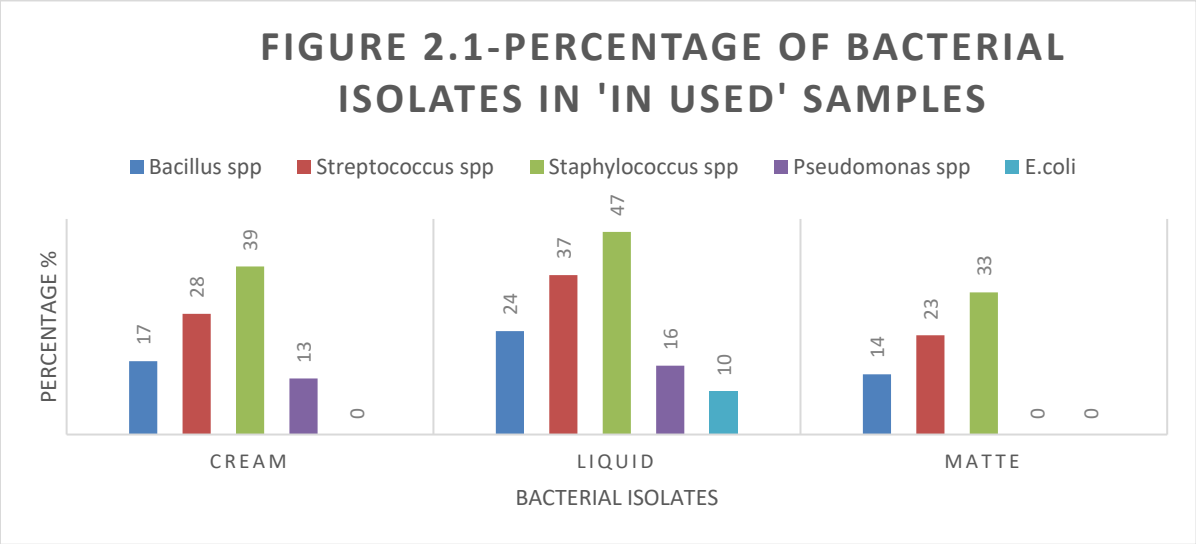


Figure1.1 and 1.2 represents percentage of fungal and bacterial isolates in expired lipsticks



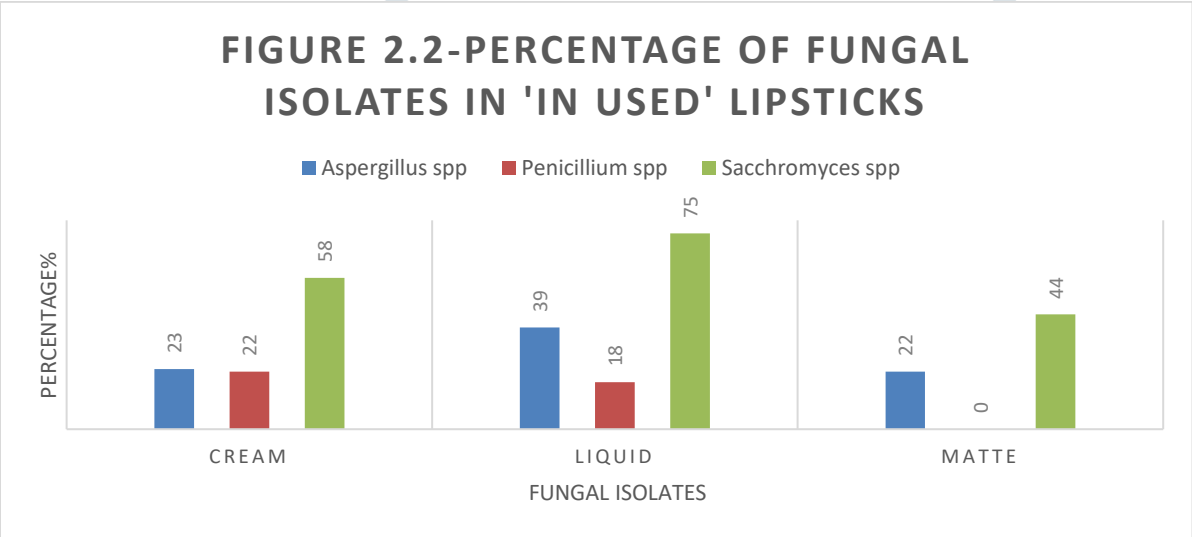


Figure 2.1 and 2.2 represents percentage of bacterial and fungal isolates in ‘in used’ lipsticks

Conclusion

Lipsticks contains preservatives but sometimes are still subjected to microbial contamination. This may occur due to humid environment, unhygienic handling and aging of product. Expired lipsticks were found to be more prone to bacterial contamination than fungal contamination. Expired matte lipsticks showed least, cream lipsticks showed moderate and liquid lipsticks showed highest degree of microbial contamination. It was observed that 'in used' lipsticks are prone to both bacterial and fungal contaminations. The 'in used' liquid lipsticks showed highest, cream lipsticks showed moderate and matte lipsticks showed least total bacterial and total fungal count. Contamination by pathogenic and antibiotics resistant bacterial isolates were observed in liquid lipsticks which may be due to unhygienic handling and exposure to contaminated environments like washrooms. From this study it can be concluded that matte lipsticks are least prone to microbial contamination when compared to cream and liquid lipsticks.

References

1. Dadashi L, Dehghanzadeh R. 2016. Investigating incidence of bacterial and fungal contamination in shared cosmetic kits available in the women beauty salons. Health Promot Perspect. 6(3):159-163. doi: 10.15171/hpp.2016.25.
2. Ezenna *et al.*2017. Spoilage Bacteria Associated with Selected Body Lotions Commonly Used amongst Students of the University of Port Harcourt, Nigeri. JPRI, 19(5): 1-7, Article no. JPRI.33478
3. Johnson R. 1999. 'WHAT'S THAT STUFF?' Chemical & Engineering News
   Copyright © 1999 American Chemical Society. July 12,1999 Volume 77, Number 28 CENEAR 77 28 p.31 ISSN 0009-2347
4. Orus, P. and S. Leranoz. 2005. Current trends in cosmetic microbiology. International Microbiology, 8: 77-79
5. Sahahat Saeed and Khizran Asif. 2011. 'Bacteriological Analysis Of Lipsticks' RADS - Vol 2 (1); 21-26