Microbial contamination of eye make up product: Herbal Mascara a concern

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Abstract- Eyes are one of the most important features when it comes to beauty. Various cosmetics are used to enhance their level of attractiveness like mascaras, eyeliners, eye shadow among many more. Out of these above products mascara is the one which amplifies the eyelashes to look thick and long thus providing people with glamorous look to the eyes. But these cosmetic products must be safe to use by the consumers. In our study different brands of 15 mascara samples were randomly purchased from various cosmetic shops from suburbs of Delhi and examined microbiologically as per Indian Standard on cosmetics. Quantitativeresults of total microbial count ranged from 10^2 to 10^8cfu g^{-1}, whereas, in case of Yeast and Mould it was from 60 to 10^7cfu g^{-1}. Presence of microbial contamination in all examined mascara samples was found to be very high as compared to fungal count. Total microbial count, yeast & mould count exceeded the expected standard by considerable margin. Out of the 15 samples *Pseudomona-saeruginosa* was found in 6 samples whereas *Staphylococcus aureus* was found in 4 samples. However, none of the mascara samples were contaminated with *Escherichia coli*. The presence of *Pseudomonas aeruginosa*, *Candida albicans and Staphylococcus aureus* was found to be 40%, 47% and 27% respectively. *Staphylococcus aureus* is an organism associated with common infections caused due to any damage to the eye balls. Whereas, *Pseudomonas aeruginosa* is one of the most common agent in eye infections like conjunctivitis, keratitis and opthalmitis. Contamination in mascara may be a result of poor hygiene, contaminated raw materials or the susceptibility of the ingredients present in the cosmetic eye preparations.

Key Words: Mascaras, Microbial Contamination, Pathogens.

Introduction

According to The Federal Food and Drug Cosmetic Act criteria (2015), cosmetic means the articles intended to be rubbed, poured, sprinkled, or sprayed on, introduced into or otherwise applied to the human body or any part thereof for cleansing, beautifying, promoting attractiveness or altering the appearance and articles intended for use as a component of any such articles;except that such term shall not include soap(U.S. Food and Drug Administration (FDA), "The Federal Food and Drug Cosmetic Act Criteria" 2015).Beauty products should be easy to use, effective and safe. Make-up products, especially those used in the eye area maytrigger a number of allergic and infectious reactions. The mainadverse effects include contact dermatitis by irritation, allergiccontact dermatitis and contact dermatitis by photosensitivity (photo toxicity), which may start an inflammatory

reaction (Draelos*et al*, Saxena*et al*, Guin*et al*, Loden, *et al*, Biebl et al,Castanedo-Tardan*et al*). The cosmetics used on the ocular region arethe main cause of eyelid dermatitis, due to their pigments, resins, preservatives and vehicles (Draelos*et al*).

Mascara is one of the most popularcosmetic products, used to lengthen eyelashes and make themthicker, highlighting the feminine face (Rieger*et al*). However, this productis at a greater risk of contamination, being an aqueous-based formulation (Draelos*et al*). Also, the way it is handled may play a role in contamination, because of the greater chance of bacterial depositsoriginating from the environment and from the surface of the eyelashes making the product more susceptible to infections.

Many people use cosmetics unaware of the dangers that can threaten their health from their usage. From previous studies, researchers concluded that cosmetics such as eye makeup have the ability to induce microbial growth and possibly cause infections. Microorganisms can grow on almost every substances existing in nature and often able to attack or even decompose them, cosmetic ingredients are rich in nutrients that provide organic substrates in the form of sugar, starch, protein, amino acids, organic acids, alcohols, lipids etc. for microbial growth (Franca *et al*), addition to that ,water is a fundamental requirements for any microorganisms likely to contaminate the cosmetics products , thus untreated or non sterile water can support microbial growth leading to contamination of cosmetics products (Luis *et al*), generally microorganisms of interest in raw materials or cosmetic products grow best around neutral pH 7.0 and many yeast and molds are able to tolerate acid pH conditions(Razooki*et al*). To avoid microbial contamination of cosmetics during use and storage, the manufacturers add preservatives to their products. Laboratory evaluation of the effectiveness of the preservatives in mascaras by the usual microbiological procedures is difficult as many formulations are not readily solubilized by water (Ahearn*et al*).Antimicrobial preservatives are substances added to dosage forms to protect them from microbial contamination. However, inmany cosmetics no expiry date has been reported and may lose the preservativeactivity and became a potential risk for microbial contamination. But two different problems arise when preservatives are used in cosmetics, first are that micro-organisms can easily contaminate the cosmetics when the amounts of antimicrobial agents are kept low for safety and economy, and second are that serious problems of skin reactions produced by antimicrobial agents are caused when their amounts are increased for preventing microbial contamination. Some of the microbes like *Staphylococcus sp., Corynebacterium sp., Pneumococcus sp.,* are commonly found on or near the eye. *Staphylococcus epidermidis* and *Staphylococcus aureus* proliferate in contaminated mascaras.

The most common infections caused bythese microorganisms occur especially when the surface of the eyeballis damaged, in other words, traumatized (Draelos*et* al). *Pseudomonas aeruginosa*is the main agent of eye infections like conjunctivitis, keratitis and ophthalmitis, which may threaten the integrity of theeye, destroying tissues and damaging visual acuity (Esteva*et al*). Infections by *P. aeruginosa* have been reported to occur due to contaminated mascara, trauma to the eye or bad hygiene (O'Donoghue*et al).* Fungi also befound in contaminated mascaras, although less frequently thanbacteria, being related to immune-compromised people or thosewho wear contact lenses (Draelos*et al*, Esteva*et al*). Similar study also reported more of bacterial than fungal contamination (Nasser *et al*).

The quality and performance requirements for mascara are asfollows: They should (i) be non-irritating as they are applied soclose to the eyes, (ii) go on evenly and not harden the eyelashes orform blobs, (iii) make the eyelashes look thick and long, (iv) makethe eyelashes curl effectively, (v) have an appropriate lustre, (vi)have an appropriate drying time, (vii) not go on to the lower eyelidswhen dry and their appearance must not be spoiled by sweat,tears or rain, (viii) be easy to remove, (ix) be easy to use throughout their period of use, (x) not be contaminated by microorganisms(Mitsui *et al*).

The study involves: a) Collection of mascara samples from different location b) Enumeration of total aerobic microbial count as well as total yeast & mould count c) Isolation and identification of *Escherichia Coli*, *Pseudomonas aeruginosa*, *Staphylo coccus aureus* and *Candida albicans* from mascara samples d) Interpretation of the results for the benefit of human welfare by increasing general awerness among the people.

Material and Methods

Collection of mascara samples

Fifteen samples of Mascara (Fig. 1) were collected from different markets in Delhi region (Table-1). The samples were analyzed for microbiological parameters including total aerobic microbial Count, total yeast and mould Count,isolation and identification of pathogenic microorganisms like *Pseudomonas aeruginosa*, *Escherichia Coli, Staphylococcus aureus* and *Candida albicans* following respective "Indian Standard for Microbiological Examination of Cosmetics and Cosmetic Raw Materials".The materials and methods required for this study are listed here in accordance to their source of availability and grades:

Figure-1 Mascara samples taken from the Delhi market

Table-1 Sample collection from different location

S.No	Sample code	Name of location
1.	MK-1	Malkaganj, Delhi
2.	MK-2	Faridabad, Delhi
3.	MK-3	Tilak Nagar, Delhi
4.	MK-4	Paharganj, Delhi
5.	MK-5	Paharganj, Delhi
6.	MK-6	Paharganj, Delhi
7.	MK-7	Paharganj, Delhi
8.	MK-8	Paharganj, Delhi

9.	MK-9	Paharganj, Delhi
10.	MK-10	Mayapuri, Delhi
11.	MK-11	Paharganj, Delhi
12.	MK-12	Mayapuri, Delhi
13.	MK-13	Mayapuri, Delhi
14.	MK-14	Mayapuri, Delhi
15.	MK-15	Mayapuri, Delhi

Enumeration of Total Aerobic Microbial Count (TAMC)

1:10 dilution was prepared after homogenization of the sample. Then this was serially diluted up to 10^{-6} dilutions. 1ml of each dilution was transferred into sterile petri dishes. Molten media of Soyabean Casein Digest Agar was poured onto the respective plates as per Indian Standard and then incubated at 32^0C for 5days.After incubation the plates were observed for the bacterial colonies with the help of colony counter and then calculated as colony forming unit (cfu)/gram.

Enumeration of Total Yeast & Mould Count (TYMC)

1:10 dilution was prepared after homogenization of the sample. Then this wsa serially diluted up to 10^{-6} dilutions. 1ml of each dilution was transferred into sterile petri dishes. Molten media of Sabourauds Dextrose Agar was poured onto the respective plates as per Indian Standard and then incubated at 22^0C for 7days.After incubation the plates were observed for the bacterial colonies with the help of colony counter and then calculated as colony forming unit (cfu)/gram.

Isolation and identification of Pathogens

Detection of *Escherichia coli*

For the detection of *E.coli* approximately one gram was added in 9ml of Soyabean Casein Digest Broth and incubated at 32^0C for 48 hours. Further subcultured on Mac Conkey Agar plates and incubated at 32^0C for 48 hours. Plates was observed for appearance of pink colonies.Further confirmation done by gram staining and biochemical tests as per Indian Standard.

Detection *Staphylococcus aureus*

For the detection of *S. aureus* approximately one gram was added in 9ml of Soyabean Casein Digest Broth and incubated at 32^0C for 24 hours. Further subcultured on Baird Parker Agar plates and incubated at 32^0C for 72 hours. Plates was observed for appearance of black colonies with grey margin and clear halos. Further confirmation done by gram staining and biochemical tests as per Indian Standard.

Detection of *Pseudomonas aeruginosa*

For the detection of *P. aeruginosa* approximately one gram was added in 9ml of Soyabean Casein Digest Broth and incubated at 32^0C for 24 hours. Further subcultured on Cetrimide Agar plates and incubated at 32^0C for 72 hours. Plates was observed for appearance of fluorescent colonies.Further confirmation done by gram staining and biochemical tests as per Indian Standard.

Detection of *Candida albicans*

For the detection of *C. albicans* approximately one gram was added in 9ml of Sabouraud Dextrose Broth and incubated at 72^0C for 24 hours. Further subcultured on Sabouraud Dextrose Agar plates and incubated at 32^0C for 72 hours. Plates were observed for appearance of white creamy colonies.Further confirmation done by gram staining and biochemical tests as per Indian Standard.

Results and Discussion

Highlighting and emphasizing the eye has been possible with a wide variety of eye

cosmetics available. They include eye shadow, under eye concealers, eyeliners, mascaras, artificial eyelashes, eyebrow pencils (Draelos*et al*). Mascara has been the oldest and most commonly used option. This usually contains a mixture of waxes and pigments in addition to resins or petroleum distillates (Fagien*et al*). Effects of mascara are temporary. Risk of microbial contamination is always a problem. Contamination of microorganisms in cosmetics may cause spoilage of the product and when pathogenic they represent a serious health risk for consumers (Campana*et al*).Microbial contamination of cosmetic products is a matter of a great importance to the industry and it can become a major cause of both product and economic losses. The need of the microbial quality of cosmetics is well-clarified and well recognized.

Therefore, this study is aimed to evaluate the cosmetic products according to their microbial contents. Results of this study reflect the urgent need to reassess our methods to control the microbial contamination of cosmetics eye preparations. The results showed that Mascara an eye cosmetic preparation, when testedfound contaminated in varying degrees including bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* whereas all tested preparations were free from *Escherichia coli*. The total microbial count (Fig 3A &3B) of all detectedbacteria are ranging from 10^2- 10^8 cfu $g^{-1}$and yeast and mould count (Fig 3C &3D) in the range of 60- 10^7cfu g^{-1} (Table 2). It was observed in the present study that both total microbial count and yeast & mould count of Mascara were not in compliance with the specified requirements of the standard. However, presence of microbial contamination in all examined mascara samples was found to be very high as compared to fungal count.Fig 2 represents the range of TAMC & TYMC. Fungi can also be found in contaminated mascaras, although less frequently than bacteria being related to immune-compromised people or those who wear contact lenses [Esteva*et al*].

The study results revealed the drastic contaminating level of yeast and mould in six Mascara samples MK-1, MK-3, MK-4, MK-5, MK-6 and MK-11 which were in the range of 10^4-10^7cfu. g^{-1} whereas no yeast & mould was found in samples MK-2, MK-10, MK-12, MK-14 and MK-15.

Table-2 Microbiological profiling of Mascara Samples

Sample code	Total Aerobic Microbial Count cfu g^{-1}	Total Yeast & Mould Count cfu g^{-1}	*E.coli*	*P. aeruginosa*	*S.aureus*	*C.albicans*
MK-1	$1.0x10^7$	$9.0\ x10^5$	Absent	Present	Absent	Absent
MK-2	$2.0x10^7$	Less than 10	Absent	Absent	Absent	Absent
MK-3	$1.0x10^7$	$8.0x10^4$	Absent	Absent	Absent	Present
MK-4	$3.0x10^6$	$1.3x10^5$	Absent	Present	Absent	Present
MK-5	$2.2x10^5$	$3.0x10^5$	Absent	Absent	Present	Present
MK-6	$3.2x10^7$	$1.5x10^6$	Absent	Present	Present	Present
MK-7	$5.6x10^4$	65	Absent	Absent	Present	Absent
MK-8	$2.1x10^4$	60	Absent	Present	Present	Present
MK-9	$1.2x10^7$	$1.6x10^3$	Absent	Present	Absent	Present

MK-10	6.8 x 10^2	Less than 10	Absent	Absent	Absent	Absent
MK-11	1.8x10^8	3.7x10^7	Absent	Present	Absent	Present
MK-12	2.9x10^2	Less than 10	Absent	Absent	Absent	Absent
MK-13	8.6x10^2	9.2x10^2	Absent	Absent	Absent	Absent
MK-14	Less than 10	Less than 10	Absent	Absent	Absent	Absent
MK-15	1.2x10^2	Less than 10	Absent	Absent	Absent	Absent

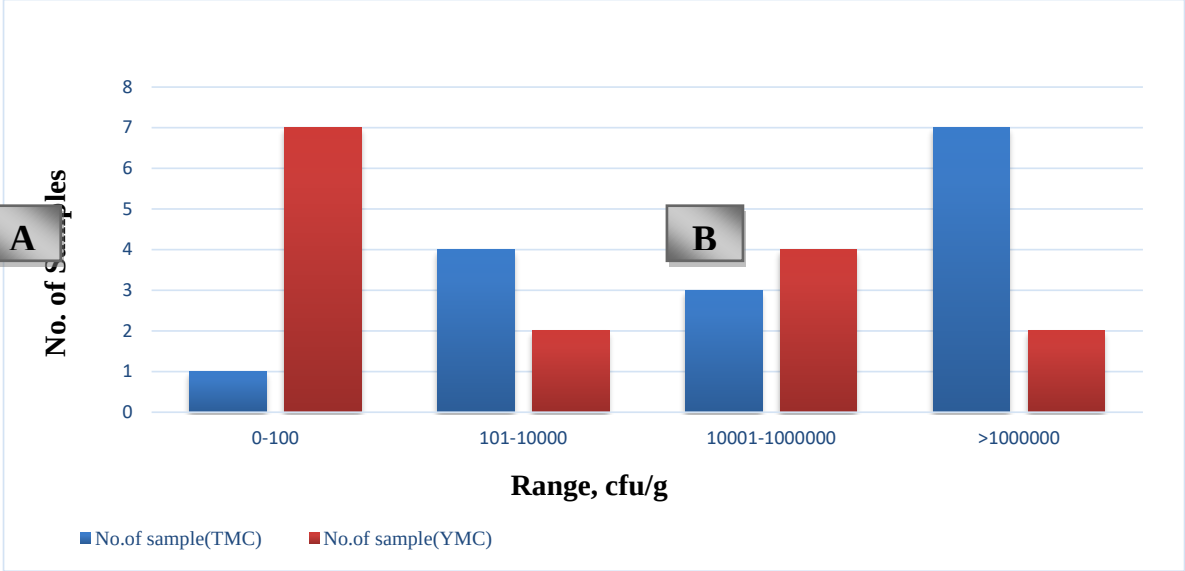

Figure- 2 Graphical representation of TAMC &TYMC in Mascara samples

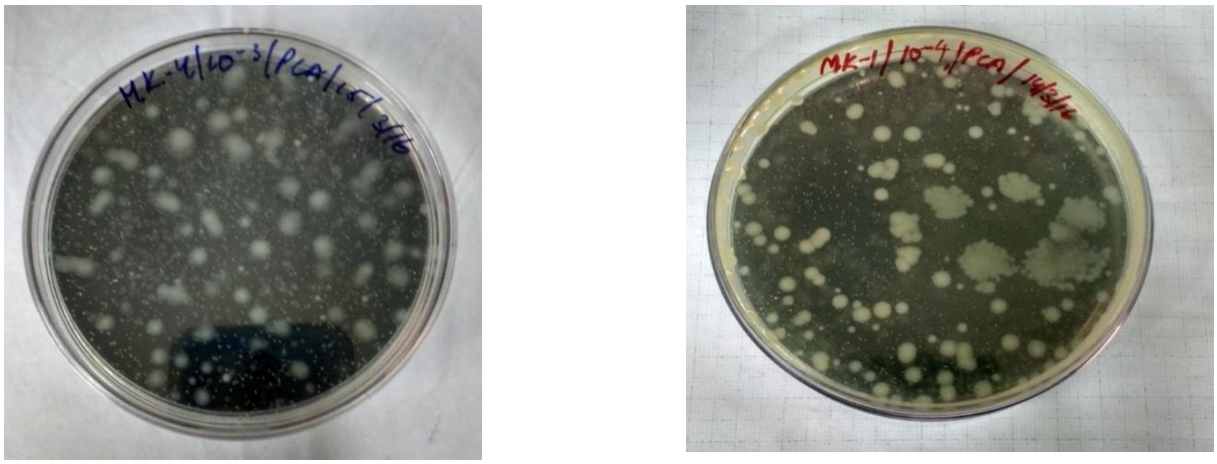

Figure- 3A and 3B Bacterial Colonies observed on Plate Count Agar

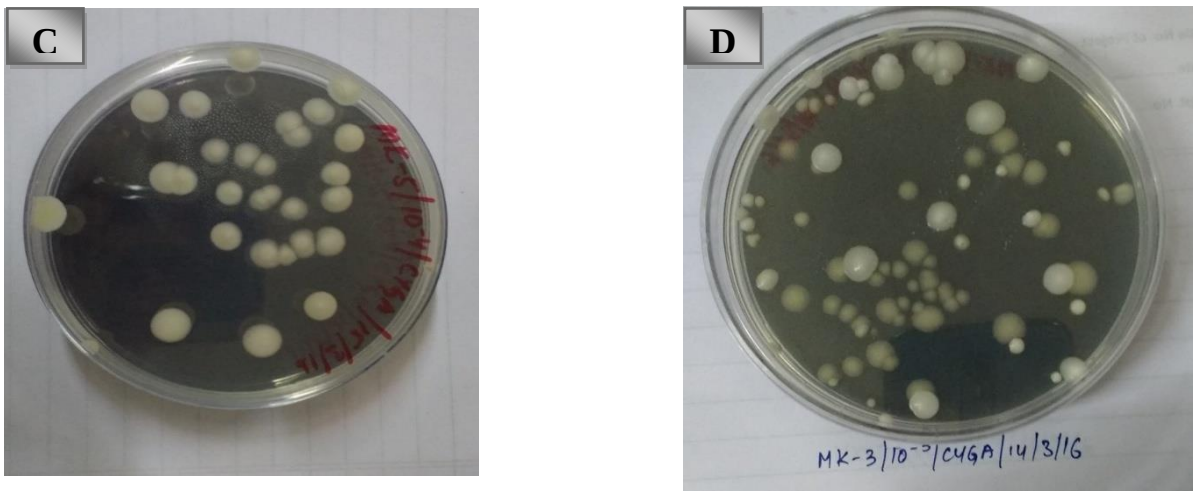

Figure- 3C and 3D Fungal Colonies observed on Chloramphenicol Yeast Glucose Agar

Table-3 Microbiological Limits of Eye products as per IS 14648-2011

Products	**Parameters**	**Limits**
Eye products (Products to be used in and around the eyes)	Total Microbial Count, cfu g^{-1}	100 (Max.)
	Yeast & Mould count, cfu g^{-1}	100 (Max.)
	Specified pathogens: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*and *Candida albicans*	Absent

Total aerobic microbial count, yeast & mould count exceeded the expected standard by considerable margin reflected in Table 2. The recommended microbiological standard for eye products (Mascara) is shown in Table-3 Biochemical characterization for identification of *Staphylococcus aureus* and *Pseudomonas aeruginosa*were shownin Table-4 and Table-5 respectively.

Table -4 Biochemical characteriztion of *Staphylococcus aureus*.

Sample Code	**Gram Staining**	**Catalase Test**	**Coagulase Test**
MK-5	Gram positive cocci in clusters	+	+
MK-6	Gram positive cocci in clusters	+	+
MK-7	Gram positive cocci in clusters	+	+
MK-8	Gram positive cocci in clusters	+	+
Positive Control *S. aureus* ATCC 6538	Gram positive cocci in clusters	+	+

Table-5 Biochemical characterization of *Pseudomonas aeruginosa*.

Sample Code	**Gram Staining**	**Oxidase Test**	**Catalase Test**	**Growth On SMA**	**Hugh Leifson Test**	**Gelatin Liquefaction Test**	**Nitrate Test**	**Starch Hydrolysis**
MK-1	Gram negative rods	+	+	+	+	+	+	-
MK-4	Gram negative rods	+	+	+	+	+	+	-
MK-6	Gram negative rods	+	+	+	+	+	+	-
MK-8	Gram negative rods	+	+	+	+	+	+	-
MK-9	Gram negative rods	+	+	+	+	+	+	-
MK-11	Gram negative rods	+	+	+	+	+	+	-
Positive Control *P. aeruginosa* ATCC 9021	Gram negative rods	+	+	+	+	+	+	-

(+) : Positive (-) : Negative

The microbial contamination in mascara sample indicates higher percentage of *Pseudomonas aeruginosa*, *Staphylococcus aureus*and *Candida albicans* showing an alarming situation for the cosmetic users. Our study revealed that out of 15 Mascara samples 9 samples were found to be contaminated with pathogenic micro-organisms. Out of all the samples studied it was found that 40% were contaminated with *Pseudomonas aeruginosa*, 27% with *Staphylococcus aureus*and 47% with *Candida albicans* whereas *Escherichia coli* were not detected (Fig 4). Although in six Mascara samples i.e. MK-2, MK-10, MK-12, MK-13, MK-14 and MK-15 no specified pathogen was detected (Table 2). According to this study, the results obtained showed that the presence of such higher counts and pathogen could lead to serious eye infections, damage of eye ball (Donoghue *et al*).

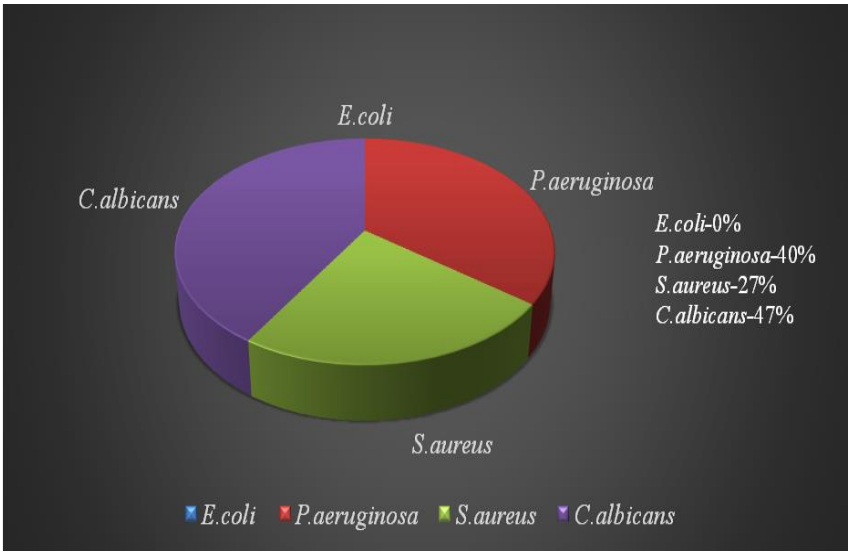

Figure-4 Percentage of pathogens in Mascara sample

The results of this study highlighted that microbiological contamination is a serious problem. Considering the risks generated by both the in appropriate usage and the possibility of in advertently using these products. There should be a public health campaigns warning of the need for the proper usage of these eye products. The contamination arises mainly because the consumers donot take proper care of the product or there is the lack of adherence to the requirements on the part of the industries that manufacturethe packaging.This may be a result of poor manufacturing practices, poor hygiene, contaminated raw materials or the succeptibility of the ingredients contained in the cosmetic eye preparation. Therefore, good manufacturing practices and hygiene must be carried out by the manufactures and personnel. Water must be tested continuously for microbial growth and raw materials should be tested before use specially those of natural origin and cosmetic eye preparation should be stored in an aseptic environment to avoid contamination before vending in the mark.

Conclusion and Recommendations

Microbiological safety is one of the most dynamic and critical parameter regarding quality of cosmetics. From the study conducted on Mascara samples it was found Total Microbial Count and Yeast and Mould count, exceeded beyond prescribed limits by Indian Standard on cosmetics. Moreover, presence of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and*Candida albicans*

showed non-compliance of this product.In order to achieve of having safe cosmetics, there should be cooperative efforts from manufacturers, health authorities and consumers.

Channels between the quality controllers and the manufacturers should always be open to improve and check the conditions for the production of cosmetics. The consumer's role can be summarized in the following steps: the instructions recommended by the manufacturer should be followed carefully, good storage conditions should be established for the products during use and skin surfaces should be cleaned before and after using eye cosmetics. Therefore, good manufacturing practices and hygiene would be effective for the control of microbiological risks in Mascara. The nodal authorities should strictly follow the rules recommended by Drug and Cosmetic Act and should be restricted to the import and export of these cosmetic products causing health related problems.

Disclaimer Statment

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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