Int. J. Ayur. Pharma Research, 2014; 2(3): 33-43 **ISSN: 2322 – 0910**

International Journal of Ayurveda and Pharma Research

Research Article

A STUDY ON THE DISTRIBUTION AND ABUNDANCE OF NORMAL FLORA ON THE HUMAN SKIN AND ITS RELATIONSHIP TO THE USE AND NON-USE OF COSMETICS

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Received on: 29/05/2014 Revised on: 03/06/2014 Accepted on: 12/06/2014

ABSTRACT

The long term use of cosmetics modifies the microbial ecology on the skin surface. The present study quantitatively assayed the microbial flora on female respondents (age group of 18-20 yrs) using and not using cosmetics, plus those who use coconut oil alone on the skin surface. The results showed that the higher number of microbial flora is observed on skin surface of respondents using coconut oil (90.4 x 10^2 cfu/ 5 cm^2 skin area). The microbial count of respondents using and not using cosmetics were 34.4 x10^2 and 45.6 x10^2 cfu, respectively, per 5 cm^2 area of skin surface. Cosmetics (C1 to C6) were assayed for antibacterial activity where C1, C2, C3, C4 and C6 were resistant to the three species of test bacteria namely *Staphylococcus aureus*, Coagulase negative *Staphylococcus* and *E. coli*. The sample C5 exhibited a low level of antibacterial activity against *E. coli* with a diameter of zone of growth inhibition of 9 mm. An assay carried out for a period of six months to compare the modifying effect of cosmetics on skin microflora of a respondent whose left hand was applied with a body lotion (C7) and her right hand not applied with any topical applicants. There was reduction in the number of microflora on the left hand with an average count of 13.3 x 10^2 cfu/ 5 cm^2 area on the skin surface, than on the right hand with an average count of 22.6 x 10^2 cfu/ 5 cm^2 area on the skin surface.

KEYWORDS: Cosmetics, Human skin, Microflora, Antimicrobial activity.

INTRODUCTION

Skin is the largest organ in human body. It contains a number of glands, which release their secretions on the skin. The skin surface is constantly fed with the secretions from sweat and sebaceous glands[1]. The population of microorganisms routinely found growing on the body surface of healthy individuals is called normal flora[2]. The normal flora includes non pathogenic commensals (not harmful to the host) or those with mutualistic existence [3]. The skin has transient, temporary and resident microflora- the three broad groups of human skin microflora which forms an integral component of normal human skin[4]. Majority of resident microflora are gram-positive bacteria that reside on the skin surface and in the follicles [5]. The human skin microflora has both beneficial and detrimental effects [4, 6-14].

The skin microflora exists in equilibrium with the host tissue and form an integral component of the normal human skin. The resident microflora develops a niche on the skin thereby helping the host against attack of harmful and infections micrograms[5] and maintenance of immune mechanisms. The microflora does not pose any problems to a healthy host. Ohalete et al have reported higher number of skin microflora among younger respondents than older ones[15]. This could be attributed to the high metabolic rate and sebum production in the younger population. These higher rates of metabolic activities and secretion levels increase the feed stock of the microorganisms on the skin surface, ultimately resulting in the greater number of microbial

flora. Colins[16] and Korting et al[17] have also observed concomitant results.

Cosmetic products are designed to bring about specific effects on the skin. The long-term use of topical products may modify or alter the skin environment, ultimately affecting the ecology of the resident microflora on human skin. The formulations of some cosmetics contain antimicrobial agents or antibiotics. Prolonged use of such products may develop resistance in the resident microflora[5,15]. The use of antiseptic and medicated soaps and other cosmetics such as deodorants and body sprays protect the skin by controlling the microorganisms[15].

It has been reported that deodorants have a bacteriostatic effect on skin microflora [15]. In their study Ohalete et al [15] observed a positive correlation between body odor and prevalence of skin flora and also to the use and non-use of deodorant. According to Paulson et al the combination of alcohol with an antimicrobial or plain lotion enhances the antimicrobial activity as a result of the synergistic effect[18]. Spray deodorants have greater antimicrobial effect than the gel and stick types[15]. This could be attributed to the alcohol base in spray type deodorants. Alcohol and propellants like butane or propane provides better results, probably due to the better and exaggerated exposure of microbial organisms to the antimicrobial agents and also due to their better penetration into fat/sebaceous glands, skin, ducts, dead cells and follicles. Nakane et al have also reported the bacteriostatic effect of zeolite incorporated deodorant for axillary odor[19].

There are only limited experiments specifically designed to determine effect of cosmetics on the human skin microflora. The present study tried to focus on the variance of skin microflora, quantitatively, among the cosmetic users and non-users of age 18-20 years, residing in Kottayam District, Kerala, India. The study also tried to assay the antibacterial activity of few cosmetics on selected microorganism. A study on the periodic modification of microbial ecology of skin microflora with continuous use of cosmetics was attempted.

MATERIALS AND METHODS

Respondents Participated in the Study

The present study evaluated the influence of cosmetics on the human skin microflora. The respondents belonging to the age group of 18-20 years, who constitute the major consumers of variety of cosmetics, were employed in this study. The respondents were classified into 3 groups: (i) those who are long term consumers of cosmetics and named R1, R2, R3, R4 and R5 respectively, for the study, (ii) those who use only coconut oil and named R6, R7, R8, R9 and R10 respectively, for the study, and (iii) those who have not used any kinds of topical agents on the skin surface so far and named R11, R12, R13, R14 and R15 respectively, for the study. The skin microflora of the five respondents from these three groups was quantitatively assayed by standard plate count using spread plate method. R16, a respondent not applying any topical agents on her skin surface, was voluntarily selected for carrying out the comparative assay of influence of cosmetics on skin microflora.

Collection of Skin Microflora from the Respondents

The skin microflora of the respondents were cultured and quantitatively assayed. An area of skin of 5 cm^2 on the left hand below the elbow region of the respondents, parallel to the body, was marked properly. The marked area was washed with free flowing sterile water. Sterile cotton swabs soaked in saline were rubbed back and forth on the marked area to collect microorganisms from the skin surface. The microorganisms collected on the swab were immediately suspended in 9 ml sterile saline and were used for preparing serial dilutions.

Serial Dilution

The skin microflora isolated from the arm region of the respondents was serially diluted using the procedure of Aneja [20]. The dilutions were prepared by suspending the microorganisms from the skin, collected in sterile cotton swabs, in 9 ml sterile saline and mixed well to obtain the 10^{-1} dilution. One ml of the suspension was transferred to 9ml saline and mixed well to obtain 10^{-2} dilution. From 10^{-2} dilution, 1 ml of the suspension was transferred to 9 ml saline and mixed well and was labeled 10^{-3} dilution.

Culturing of Skin Microflora

The microorganisms were grown on nutrient agar plates for the quantitative assay. The skin microflora of the respondents was enumerated using standard plate count method employing the spread plate type of bacterial culture. From the diluted suspensions 0.1 ml of each of 10^{-1}, 10^{-2} and 10^{-3} dilutions were added on to the agar surface and spread evenly using an L-shaped sterile glass rod. The plates were incubated at 37°C overnight. The colonies developed on the plate for each dilution were counted and recorded.

Antibacterial Activity of Cosmetics

In this study the antibacterial activity of few cosmetics available in the open market were assayed against 3 bacterial species.

Bacterial Stains Used

Bacterial cultures used in this study were obtained from the culture collections of Dianova Laboratories, Kottayam, Kerala, India. Bacterial cultures namely *E. coli*, *Staphylococcus aureus* and Coagulase negative *Staphylococcus* were included in this study. The bacterial strains were maintained on Nutrient Agar plates or slants and were stored at 4 °C.

Sample collection

The cosmetics used for this study were body moisturizing lotions, purchased from standard cosmetic stores in Kottayam District, Kerala, India. The batch numbers and expiry dates were noted. The cosmetics were labeled C1, C2, C3, C3, C4 and C5. A branded coconut oil advertised for application on skin and hair, was also purchased and labeled C6. A body lotion-C7- was used for the comparative study of modification of skin microflora after long term use of cosmetics, on respondent R16.

Antibacterial Assay by Disc Diffusion Method

Sensitivity Discs

Sterile sensitivity discs of 6 mm diameter were prepared from Whatman no. 1 filter paper. The discs were sterilized by autoclaving and stored at room temperature till use. The discs were soaked in the cosmetics for 10 minutes and were used for disc diffusion assay.

Disc Diffusion Method

Mueller-Hinton Agar (MHA) was used as base medium for screening of antibacterial activity. About 15 to 20 ml of MHA medium was poured into sterile Petri dishes and allowed to solidify. Pure isolated colonies of bacterial culture were inoculated into peptone water and incubated at 37 °C for 48 h and were used as inoculum for lawn culture on MHA. Using sterile cotton swab, 0.2 ml of 24 hr old culture was inoculated evenly on to the surface of MHA to make a lawn culture. For analyzing the antibacterial activity of cosmetics, the discs carrying the cosmetic samples were impregnated on the seeded agar plate (2 discs per plate). Discs alone were used as controls. The experiment was performed in duplicates. The plates were incubated at 37 °C for 24 hrs and observed for zone of inhibition of growth around the discs.

Influence of Cosmetics on Skin Microflora

To comparatively assay the effect of cosmetic and its influence on the ecology of skin microflora, a respondent (R16) belonging to the age group 18-20 years and not using any kind of topical agents on the skin surface was selected. The respondent was made to apply the cosmetic C7 only on her left hand and not on her right hand, regularly twice a day, for a period of six months. Assays were performed by enumerating the microbial count on the left hand versus the right hand, using serial dilution and spread plate method, starting from four months after the application of the cosmetic.

RESULTS AND DISCUSSION

The human skin is a habitat for many species of microorganisms that exist as commensals or normal flora on the skin surface and generally cause no harm to their host. The skin may also harbor other microorganisms which may be pathogenic, causing serious skin infections, or those which brings about adverse effects such as obnoxious body odor, dry skin and skin abrasions[15].

The present study investigated and comparatively analyzed the variations in microbial flora of three groups of respondents, those who have been long-term users of cosmetics, those who use coconut oil alone and those who do not use any kinds of topical applicants on skin. The respondents included in this study were females at an age group of 18-20, who are the largest group of users of cosmetics[15].

Enumeration of Bacteria in Skin Microflora

The bacterial count in colony forming units per ml, (cfu/ml) of the various dilutions obtained from the skin surface of the respondents who are long - term consumers of cosmetics, are summarized in Table 1.

Table 1: Microbial Count from Respondents who are Long-Term Users of Cosmetics

Respondent No.	Serial Dilutions Prepared from Skin Microflora		
	10^{-1} dilution (cfu/ml)	10^{-2} dilution (cfu/ml)	10^{3}dilution (cfu/ml)
R1	470	47	26
R2	380	22	14
R3	110	11	2
R4	445	46	29
R5	460	46	31
Average count using 10^{-2} dilution = 34.4 x 10^{2} cfu/ 5 cm^{2} area on skin surface			

The bacterial count obtained using the 10^{-2} dilutions of the respondents were used for enumerating the skin microflora. An average bacterial count of 34.4 x 10^{2} cfu /5 cm^{2} skin area was obtained for the group (i) respondents of the study, comprising of long - term users of cosmetics for topical application on skin surface.

The bacterial count obtained from the various dilutions prepared from the skin microflora of respondents using coconut oil alone for topical application is summarized in Table 2.

Table 2: Microbial Count from Respondents Using Coconut Oil

Respondent No.	Serial Dilutions Prepared from Skin Microflora		
	10^{-1} dilution (cfu/ml)	10^{-2} dilution (cfu/ml)	10^{-3} dilution (cfu/ml)
R6	340	47	18
R7	TNTC*	110	11
R8	320	32	2
R9	770	103	17
R10	TNTC*	160	16
Average count using 10^{-2} dilution = 90.4 x 10^{2} cfu/ 5 cm^{2} area on skin surface			

***TNTC – Too Numerous to Count**

The average bacterial count obtained using the 10^{-2} dilutions of the respondents were used for enumerating the skin microflora of the group (ii) respondents of the study, comprising of long term consumers of coconut oil for topical application on skin surface. An average bacterial count of 90.4 x10^{2} cfu was obtained in the standard plate count from 5 cm^{2} area on skin surface.

The group (iii) respondents comprised of those who are non-users of no kinds of topical agents on their skin surface. Here also the bacterial counts obtained from 10^{-2} dilutions were used for enumeration of skin microflora, the results of which are summarized in Table 3. The average bacterial count obtained in the standard plate count method for the group (iii) respondents was 45.6 x 10^{2} cfu /5 cm^{2} area on the skin surface.

Table 3: Microbial Count from Respondents who are Non-Users of Topical Agents on Skin Surface

Respondent No.	Serial Dilutions Prepared from Skin Microflora		
	10^{-1} dilution (cfu/ml)	10^{-2} dilution (cfu/ml)	10^{-3} dilution (cfu/ml)
R11	150	60	2
R12	130	21	7
R13	118	23	3
R14	340	70	13
R15	430	54	8
Average count using 10^{-2} dilution = 45.6 x 10^{2}cfu /5 cm^{2} area on skin surface			

The results of the present study revealed that the respondents using cosmetics had a less bacterial count on the skin surface than those who do not use any (Tables 1 and 3). The former group had an average count of bacterial flora of 34.4 x 10^2 cfu/ 5 cm^2 area on skin surface where as the latter group had a greater bacterial count of 45.6 x 10^2 cfu/ 5 cm^2 area of skin. The results obtained were as expected, except for the third group, the respondents using coconut oil on their skin surface, who had the highest number of bacteria on the skin surface, giving an average bacterial count of 90.4 x 102 cfu/ 5 cm2 skin area. The present study shows that coconut oil has a positive modulatory effect on the human skin surface. It is also evident from this study that there is a strong correlation between the numerical abundance of skin microflora and the use and non-use of cosmetics. Ohalete et al had conducted a similar study on the antimicrobial activity of deodorants on skin flora. They observed that deodorants had a bacteriostatic effect on skin microflora[15]. Ohalete et al also could observe strong positive correlation between prevalence of skin microflora and the use and non-use of deodorants[15].

Antibacterial Activity of Cosmetics

Many a cosmetics are formulated to contain antimicrobial agents or antibiotics which can effectively change the microbial ecology on application to the skin surface. This may be the reason behind the lower number of bacterial flora of cosmetic consuming participants in this study. The detrimental effect of cosmetics on the skin microflora has been proved earlier. The short term consequences of cosmetics on skin ecology may be minimal, but long term consequences may result in serious shifts in ecological balances[5]. Another significant point of concern is the generation of resistant microflora following the constant and increased use of cosmetics. Ohalete et al had isolated a total of 212 bacterial and fungal species from neck, armpit and chest of human participants, where the most prevalent bacteria had been *Staphylococcus* species and *E. coli*[15]. *Staphylococcus*, existing in the sebaceous follicles, is a major group of bacteria representing the resident flora of skin [21].

In the present study, the antibacterial activity of six cosmetic products- body lotions and one branded coconut oil (advertised for topical application on skin and hair), labeled C1, C2, C3, C4, C5, C6, respectively,- were assayed against three bacterial species viz, Coagulase negative *Staphylococcus* (a resident flora), *S. aureus* (a skin pathogen) and *E. coli*, a resident intestinal flora in humans. *E. coli* was also included in the antibacterial assay to investigate whether chance ingestion of cosmetics may alter or modify the human intestinal flora. Other than C5, neither the cosmetics nor coconut oil (C6) exhibited antibacterial activity against the test organisms. The sample C5 inhibited the growth of *E. coli* with a diameter of 9 mm of zone of inhibition of growth (Table 4). Even though C5 has lower anti-*E. coli* activity, the result indicates that there is the possibility of cosmetics modifying the intestinal microflora if ingested accidentally. A similar study by Ohalete et al revealed that deodorants inhibited the growth of *S. aureus, S. epidermidis* and *E. coli*[15]. Antimicrobial activity of those deodorants could be possibly due to the presence of alcohols or propellants like butane or propane, which are potent antimicrobial agents[18]. The present study proves the modulatory effect of cosmetics -body lotions-on the skin and intestinal microflora. The antimicrobial agents in these products may penetrate into the secretary glands, ducts, dead cells and follicles in the long run and dislodge the microbes on skin surface.

Table 4: Antibacterial Activity of Cosmetics on Resident and Pathogenic Microflora

Cosmetic Used	Coagulase negative *Staphylococcus*	*S. aureus*	*E. coli*
C1	R	R	R
C2	R	R	R
C3	R	R	R
C4	R	R	R
C5	R	R	9 mm
C6	R	R	R

Disc Diameter = 6mm

Cosmetics and their Effect in Modifying Skin Microflora

The capacity of cosmetics in modifying the skin microflora has been studied earlier [5]. A parallel study was conducted in this work to comparatively analyze the effect of cosmetics on skin microflora. A respondent (R16), not using any kind of topical agents on skin surface, was voluntarily selected and made to apply a body lotion (C7) only on her left hand till the end of the study. The opposite hand served as the control where the lotion was not applied. The microbial population on left hand after application of cosmetic was compared with that on the right hand. The bacterial count obtained on the left hand during 4th, 5th and 6th month of study was 11, 17 and 12 x 10^2 cfu/5 cm^2 area of the skin surface. The bacterial count from the right hand had been 30, 27 and 11 x 10^2 cfu, respectively, per 5 cm^2 area of skin surface during the 4th, 5thand 6th month of study. The bacterial count was more on the right hand where the cosmetic was not applied (average bacterial counts of 22.6 x 102 cfu/5 cm2 area of skin surface), than on the left hand which was treated with the cosmetic (average bacterial counts of 13.3 x 102 cfu /5 cm2 area of skin surface). This assay was conducted over a very short period of time. However, it is evident from the results that cosmetics do affect the distribution of microbial population on human skin surface. The rate of modification of microbial number and ecology may vary from person to person. This could be addressed only after a study extended over a longer period of time and also over many a number of respondents.

Table 5: Effect of Cosmetics in Modifying Skin Microflora

Respondent No.	Period of study after application of cosmetic C7	Serial Dilution of Microflora					
		Left hand: C7 Applied			Right hand: No Topical Agents		
		10-1 dilution (cfu/ml)	10-2 dilution (cfu/ml)	10-3 dilution (cfu/ml)	10-1 dilution (cfu/ml)	10-2 dilution (cfu/ml)	10-3 dilution (cfu/ml)
R16		140	11	0	190	10	3
		170	36	17	258	27	8
		113	42	4	180	11	2

CONCLUSION

It is most important that majority of the present day topical products pose serious threat to the normal flora of skin and ultimately to human health. In spite of this, the demand for cosmetics is increasing worldwide, particularly among the youth. A major lacuna in this field of study is the limited number of scientific experiments and reports addressing the effects of cosmetic products on the microbial ecology of skin. The results of the present study on the comparative analysis of influence of cosmetics in altering or modifying the ecology of human skin microflora corroborate with earlier reports and suggest a strong positive correlation between the reduction in the number of human skin flora and the long-term use of cosmetics. The least number of bacterial counts was observed on the skin surface of respondents who were long-term users of cosmetics, as expected. Also the bacterial count enumerated from the skin surface of a respondent applied with cosmetic showed considerable reduction in their number when compared to that from the same respondent on a different site where the cosmetic was not applied. The topical products available today do not pose serious threat to human health. Even then the manufactures should be encouraged and mandatorily asked to test the effect of their products on human skin flora, before introducing the product into the open market. The advance of technologies, molecular modelling, and bioinformatics, may be effectively implemented for this cause. The manufacturers or product formulators should predict outcomes - whether good, neutral or of concern.

ACKNOWLEDGEMENTS

The authors like to express their gratitude to DIANOVA Laboratories, Kottayam, Kerala, India, for providing the bacterial cultures. The authors acknowledge the co-operation and support of the respondents who had participated in the study.

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Cite this article as:
Varghese LS, Sajeevkumar A, Muralidahran AV, Paul KJ, Viswanathan S. A Study on the Distribution and Abundance of Normal Flora on the Human Skin and its Relationship to the Use and Non-Use of Cosmetics. Int. J. Ayur. Pharma Research. 2014;2(3):33-43.

Source of support: Nil, Conflict of interest: None Declared

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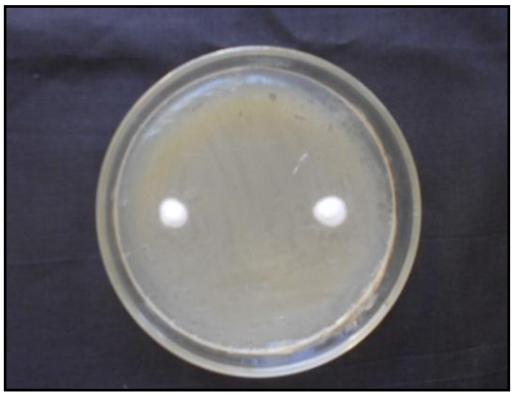
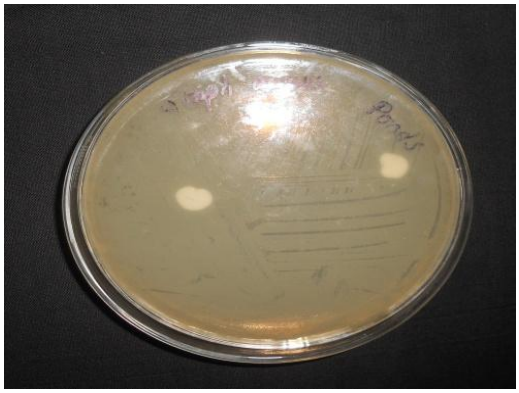
Figure 1: Antibacterial Activity of Cosmetics against *Staphylococcus aureus*



C1

C2



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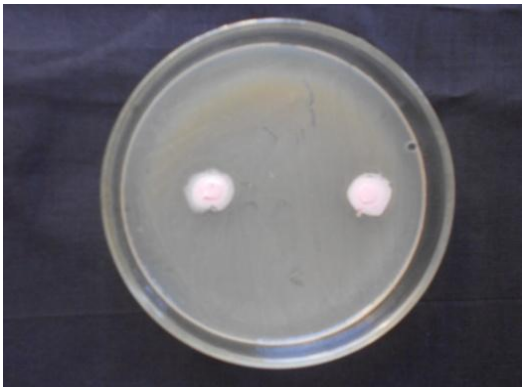

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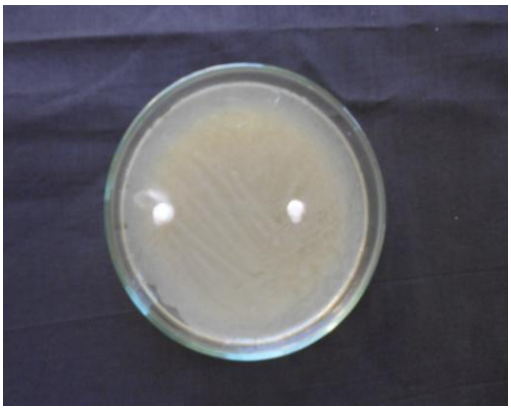

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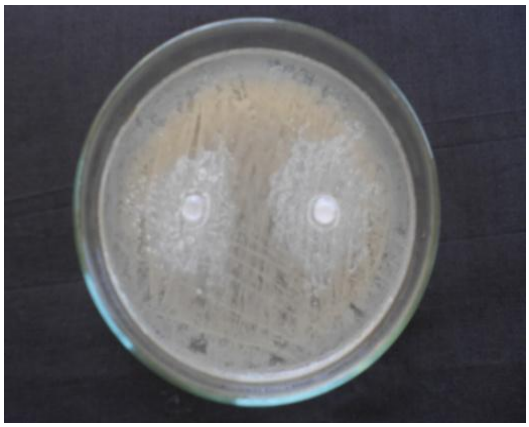
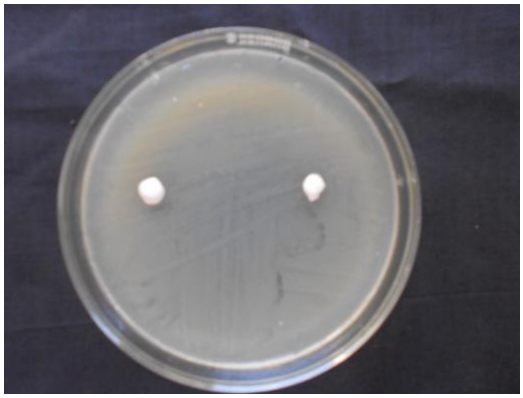


C6

Figure 2: Antibacterial Activity of Cosmetics against Coagulase Negative *Staphylococccus*



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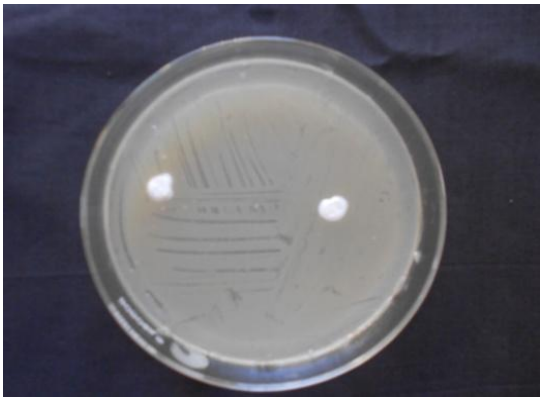

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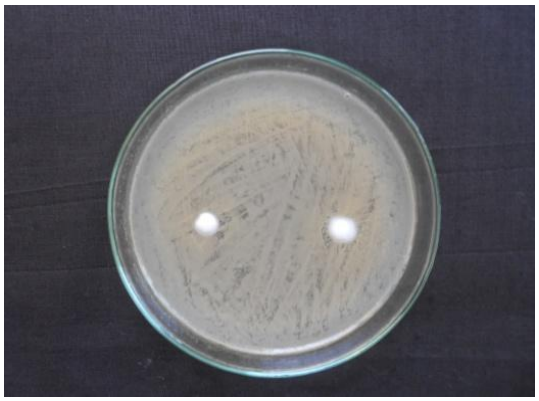

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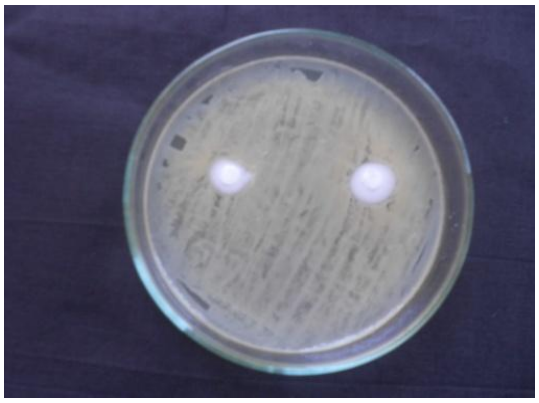

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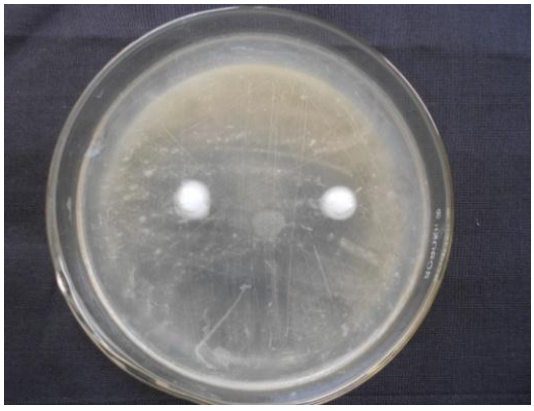
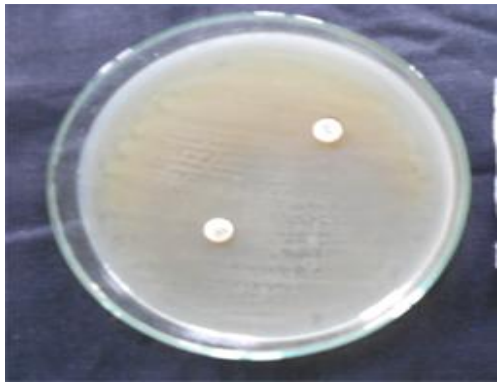


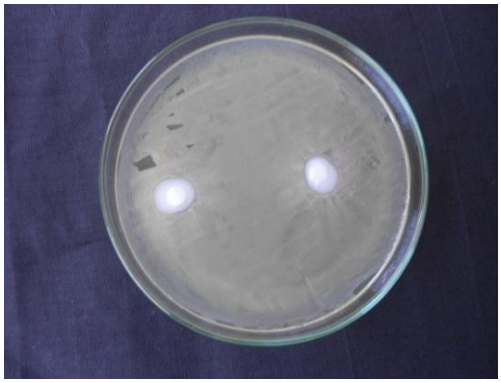
C5

C6

Figure 3: Antibacterial Activity of Cosmetics against *E. coli*



C1



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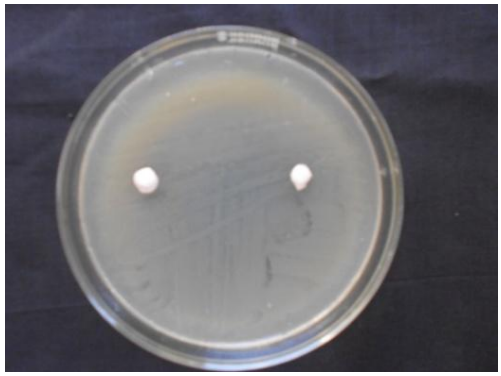
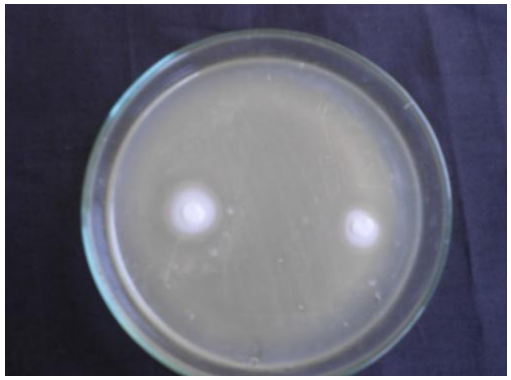
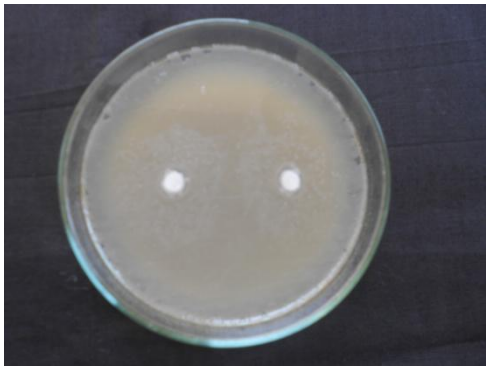


C3

C4

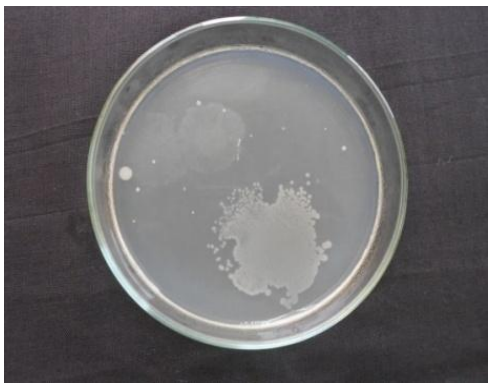


C5



C6

Figure 4: Quantitative Enumeration of Microbial Flora on 5 cm² Area of Skin Surface Obtained from the Respondents



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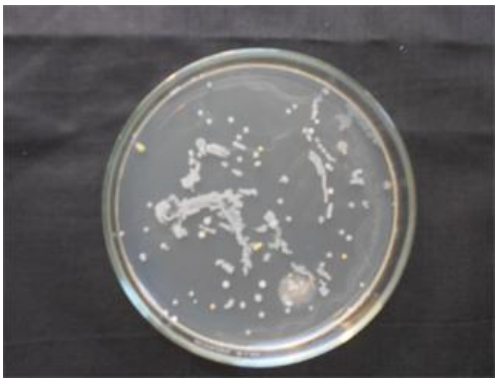
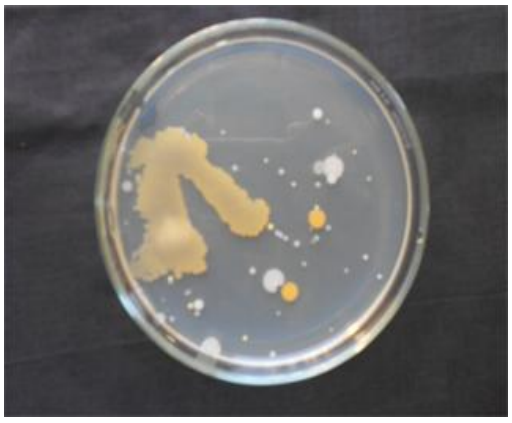
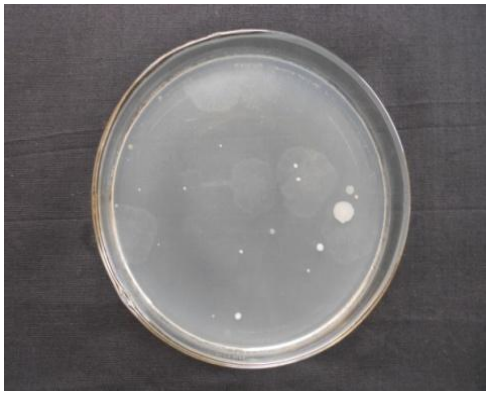


R3

R7



R9



R12

R13