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Research Article

IMPACT OF SUPERFICIAL BLENDS ON SKIN MICRO BIOTA

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ABSTRACT

Visakhapatnam, the ***City of destiny*** is accomplished with varied types of Eco systems and Environment. The humid and polluted atmospheres play a major role in shaping Visakhapatnam environs. In general, majority of the urbanites use cosmetics/personal care products like antibacterial soaps, deodorants, creams and moisturizers. Synthetic preservatives containing antimicrobial properties are used to increase the shelf life of these products. The presence of these products inhibits the growth of microbes when the cosmetics/personal care products are applied onto the body. Some of the microbes become resistant to the preservatives and in some cases newly established colonization by a typically pathogenic microbe will replace the normal microbiota. It is proved that the long term use of products containing antibiotics leads to increased numbers of antibiotic-resistant resident microbiota. This concern could be extended to chemically synthesized antimicrobials in cosmetics, since biological systems, by their very nature, are flexible and selectively adaptable. Hence there is need and necessity to know the extent of application of these products onto the body. The present study is an attempt to assess the impact of preservatives on the skin microbiota. This work certainly provides new insights in the usage of personal care products.

Keywords: Cosmetics, Personal care products, Normal microbiota, Preservatives, Antimicrobial resistant.

INTRODUCTION

Microorganisms that are always associated with the human body without causing any deleterious effects are known as normal micro biota of human body . The microbial communities present on skin are determined by the skin conditions, the host's hormonal status, age, gender, and ethnicity (Fierer N,2008;Fredricks DN,2001;Grice EA,2009;Roth&James,1988). Skin serves as a protective barrier and harbours large numbers of colonizing bacteria. Based on scientific and clinical evidence generations of dermatologists have asserted that microbes influence the natural courses of several skin diseases;*Staphylococcus epidermidis*, for instance, is frequently cultured from healthy skin and may protect humans from pathogenic bacteria . *Staphylococcus epidermidis* especially inhibits the bio film formation of *Staphylococcus aureus* and nasal colonization (Iwase T et al.,2010).Human skin can be an inhospitable environment (Cogen AL,2008) and skin disease in general can result from transformation of a resident microorganism to a pathogenic state under particular conditions or from newly established colonization by a typically pathogenic microbe (Heidi H,2011). Despite these protective mechanisms, microorganisms still survive and thrive. The irregular topography and different skin site characteristics provide distinctive habitats for bacteria. Many other elements potentially contribute to the composition of microbial communities residing on and in skin. External factors such as ambient humidity, seasonal weather conditions, previous antibiotic treatment, clothing type, use of lotions, creams, cleansers, deodorants or anti-perspirants, hygiene frequency and other environmental surfaces interact with and can influence cutaneous bacteria. Intrinsic factors such as age, genetic makeup and host immune system also influence the composition of skin microorganisms (Fierer N,2010). Among the normal microbiota, *Micrococcus lutes*, *M.varians, Staphylococci epidermidis, S.capitis, S.warneri, Corynebacterium xerosis, C.jeikinum, Brevibacterium spp.* and occasionally *Mycobacterium spp* are commonly present bacteria on the human skin. These microbes usually protect our body from invasion and colonization by foreign organisms. *Corynebacterium spp*. predominates at moist sites for instance, which corresponds with culture-based observations indicating that these organisms seem to favor skin with high humidity (Grice EA,2009;Cogen AL,2008) . The resident microflora is beneficial in occupying a niche and denying its access to transients, which may be harmful and infectious. Also, the residents are important in modifying the immune system. In the healthy host, the microflora causes few and temporary problems. Therefore, it is of interest that topical products have little or no effect on the ecology of the microflora. The short-term consequences are minimal whereas the long-term problems are difficult to predict(Keith T,2002). There is evidence that when the resident skin microflora is disturbed, as with prolonged antibiotic therapy, infections of skin can occur, eg. Gram negative folliculitis (Leyden JJ,1973). Long-term usage of antibiotics in products leads to increased numbers of antibiotic-resistant resident microflora. In essence, the pool of resistant genes increases which can be disseminated in the community through the resident microflora and onto other micro-organisms (transients) which are also infectious agents. An example would be antibiotic resistance in *S. epidermidis* transferred to *S.aureus* (Miller YW et al.,1996). This concern could be extended to chemically synthesized antimicrobials in cosmetics because biological systems, by their very nature, are flexible and selectively adaptable (Russel AD,2001). A synthetic cosmetic usually contains seven base ingredients, which are water, emulsifiers, preservatives, thickeners, colors, fragrances and stabilizers(Lalitha CH,2012). Preservatives in the product may remain active on the skin and with continued use of the product the resident microflora is altered. The risk for this happening is dependent on the residual activity of the preservatives in the skin (Russel AD,2001). The present work reports the impact of preservatives present in the personal care products; talc-cum-powder, deodorant spray and fairness cream on the skin flora. The products containing triclosan, phenoxy ethanol, parabens as preservatives were selected for application onto the human skin for testing their action on the skin microbiota. People usually spray deodorants to reduce the body odour. Majority of the body deodorants contain antibacterial substances that act selectively against gram positive bacteria to reduce the production of volatile unsaturated fatty acids and body odour. However, deodorants can shift the normal microbiota to predominantly gram-negative bacteria and precipitate subsequent infections (Prescott,2002). The inhibitory effects of the deodorants were found to be bacteristatic rather than bactericidal. Thus the microbes are likely to recover and continue to grow on the host's skin long after the deodorants have evaporated or washed away. For this reason, continues regular use of these deodorants will be helpful for effective control of skin microflora. However, the constant use of deodorants for the control of skin microbiota should be checked to avoid mutation and development or resistant strains (Davin-Regli A,2006). *Enterobacter gergoviae* collected from diverse cosmetic formulations containing parabens showed resistance in the form of efflux (Ohelate CN,2012). Triclosan is bacteriostatic at low concentrations and bacteristatic at higher concentrations. It is used as preservative in cosmetics as well as in many personal care products. Studies has been proved that *Pseudomonas aeruginosa* is highly resistant to the triclosan (Russel AD,2004).

MATERIALS AND METHODS

Personal care products

A Personal care product is a non-medicinal consumable product that is intended for topical care, grooming of the body and hair through application without affecting the structure or functions of the body. Personal care products are specifically for use in such activities as cleansing, toning, moisturizing, hydrating, exfoliating, conditioning, anointing, massaging, coloring/decorating, soothing, deodorizing, perfuming, and styling. In the present investigation three products; talc-cum-powder, deodorant spray and fairness cream were used to study the impacts of preservatives on the skin flora. The three personal care products were procured from the local markets.

Isolation and identification of skin microbiota

Samples from superficial skin were obtained by using sterile cotton swabs. The swabs should be dipped in saline solution before obtaining the sample. The donors of the facial swabbing were drawn from Visakhapatnam city. The excess saline was removed by pressing the swab against the wall of the test tube. Swabbing was done on the face for about 10secs. The swab was inoculated on the CLED agar plate. The plates were incubated in inverted position at 35° C for 24 to 48 hrs. The colonies developed were observed for the colony characters such as colour, texture and consistency. The developed bacterial colonies were subjected to Gram staining. The results were compared with the known bacteria characteristic features. All the colonies were incubated on the Nutrient agar media for 48 hrs at 37°C for pigment production, of which a few colonies were identified and isolated. The selective colonies were sub cultured in Nutrient broth. Biochemical tests such as IMViC, Catalase, Oxidase, Urease, Starch hydrolysis and fermentation tests with glucose, fructose & lactose were performed. Further, Bergey's manual of systematic Bacteriology was employed for the species level identification of the colonies(Cuppuccino G,1999).

RESULTS

The data relating to the biochemical characters of the different isolates were presented in Table 1.

The data presented in Table 1 indicate that 80% of the colonies in majority of the study samples were identified as Bacillus spp. Both *Bacillus cereus* and *B. subtilis* were identified among the Bacillus spp. In general the Bacillus spp are present on the skin as a contaminant from surrounding soil and the surrounding air (Watanable K,2003). *B. subtilis* could be expected to temporarily inhabit the skin and gastrointestinal tract of humans, but it is doubtful that this organism would colonize other sites in the human body (Edberg SC,1991). *B.cereus* also has a saprophytic life cycle in which spores germinate in soil, with the production of a vegetative bacillus, which could then sporulate, maintaining the life cycle(Arnsen LPS,2008). The remaining colonies belong to the microbiota of human skin. Among them Micrococcus spp., Staphylococcus spp., Corynebacterium spp., and Pseudomonas spp. are identified up to the species level based on the Bergey's manual of bacteriology.

Table 1: Shows Biochemical characters of different isolates

Isol-ate No.	Gram stain	Shape	ID	MR	VP	CT	CAT	OXD	URS	STA	GLC	SUC	LAC
3	+	Cocci	-	-	-	+	+	+	-	+	A	-	A
4	-	Bacilli	-	-	-	-	+	-	-	-	A	AG	A
5	-	Bacilli	-	-	+	-	+	+	-	-	-	A	-
8	-	Bacilli	-	-	-	-	+	-	-	-	-	-	-
13	+	Cocci	-	+	-	-	+	-	+	-	-	-	-
14	+	Cocci	+	-	-	-	+	-	-	-	-	-	-
31	+	Cocci	-	-	-	-	+	+	-	-	A	-	A
32	+	Cocci	-	+	-	-	+	+	-	-	-	-	-
33	+	Cocci	-	+	+	-	+	-	+	-	-	A	-
42	-	Bacilli	-	+	+	-	+	+	-	-	-	-	-
45	+	Cocci	-	+	+	-	+	-	-	-	A	A	-

Abbrevations :- '+' - positive for the test; '_' – negative for the test; #A-acid producers, G-gas producers and AG-acid & gas producers

Before and after application of the personal care products

The face area was wiped with sterile cotton and skin samples were taken using a sterile swab in a closed laboratory. The sample is streaked on the nutrient agar plate. After that a personal care product was applied on to the face area. After 30 minutes, another sample was taken beside the area from where the earlier sample was taken and inoculated on the second plate. The third sample was taken from the same face but from a different area after 30 min (i.e, 1 hr) and inoculated on the third plate. All the plates were incubated at 37°C for 24 hours and observed for the growth. 20 samples from different donors were collected by applying talc-cum powder to the left side of the face, fairness cream to the right side of the face and deo spray to the fore arm. Then the samples are collected from the respective areas from the donors, inoculated on the nutrient agar plates and tested for the change in number of colonies. The talc-cum powder, fairness cream and deo spray are selected based on the survey conducted among the teenage girls living in the urban development area of Visakhapatnam.

The data presented in Table 2 indicate that in all the samples, the number of bacterial colonies has decreased after application of the personal care products with varying count indicating the susceptibility towards the product. But with time, the number of colonies regained indicating the development of resistance to the personal care products as shown in Figure 1,2 and 3 towards talc, spray and cream respectively.

Table 2: Shows Colony count of the skin swabs after application of fairness cream, talc-cum-Powder and deodorant spray

Nutrient agar plates inoculated with the test personal care products.	No. of colonies observed*		
	Initial	30 min	1 hr
Talc-cum-powder	58	14	50
Deo spray	16	4	9
Fairness cream	20	3	8

***** Each colony is an average of 5 plates (n=5)

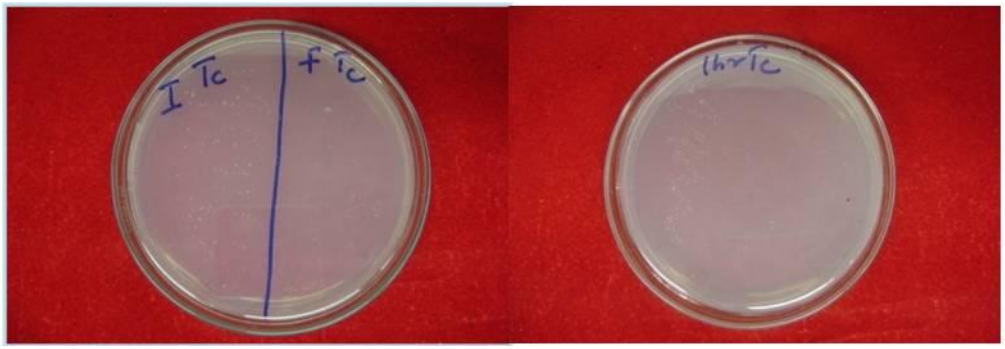

Fig. 1: Showing the growth of colonies before and after application of Talc-cum-powder

I-Colonies developed from the skin sample before applying Talc

F- colonies developed from the skin sample after 30 min application of Talc

1 hr- colonies developed from the skin sample after 60 min application of Talc

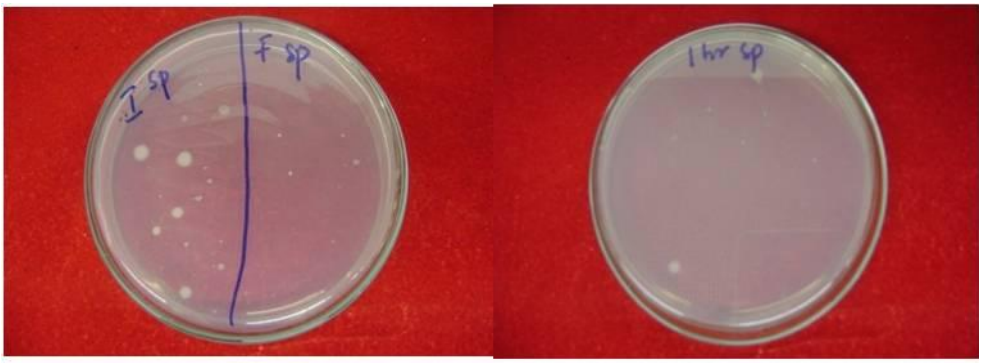

Fig. 2: Showing the growth of colonies before and after application of deospray

I-colonies developed from the skin sample before applying deospray

F- colonies developed from the skin sample after 30 min application of deospray

1hr- colonies developed from the skin sample after 60 min application of deospray

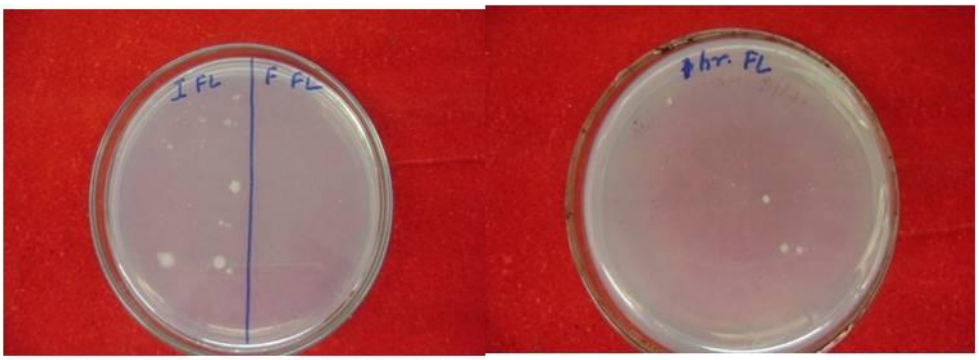

Fig. 3: Showing the growth of colonies before and after application of Fairness cream

I-colonies developed from the skin sample before applying cream

F- colonies developed from the skin sample after 30 min application of cream

1hr- colonies developed from the skin sample after 60 min application of cream

Normal-derma Experiment

The pure cultures of isolated bacteria from skin were inoculated on sterile Muller-Hilton agar plates spotted with 50µl of diluted sample of talc-cum-powder, fairness cream and deospray on sterile filter paper discs. The plates were incubated at 37^0 C for 24 hours. Zone of inhibition was observed around the discs as shown in Figure 4.

Zone of inhibition observed around F, T & S on the isolated cultures No. 2 & 11.

F- fairness cream., T- talc., S- deospray

Table 3: Response of talc-cum-powder, fairness cream and deospray towards the bacterial samples isolated from the skin.

S. No.	Zone of inhibition (in cms) on Muller-Hilton agar medium		
	Talc-cum-powder	Fairness cream	Deospray
1	1.2	≠	1.3
2	1.2	≠	1.5
3	≠	≠	0.8
4	≠	≠	≠
5	≠	≠	≠
7	≠	≠	≠
10	≠	≠	≠
11	1.0	≠	1.8
12	≠	≠	≠
16	≠	≠	≠
17	≠	≠	0.8
28	1.5	≠	≠
29	≠	≠	1.0
30	≠	≠	≠
31	≠	≠	≠
32	2.0	≠	2.0
32	≠	≠	≠
33	≠	≠	≠
34	≠	≠	≠
36	≠	≠	≠
37	≠	≠	≠
38	≠	≠	≠
39	≠	≠	≠
40	2.0	≠	1.8
41	≠	≠	≠
42	≠	≠	≠
43	≠	≠	≠
44	≠	≠	≠
45	≠	≠	≠
46	1.2	≠	≠
47	≠	≠	≠
48	≠	≠	≠

'≠' – indicates that zone of inhibition was not observed.

The data presented in Table 3 indicate that the zone of inhibition by the talc cum powder and deospray was observed in samples 1,2,11,28,32,40 & 46 and 1,2,3,11,17,29,32 & 40 respectively. Zone of inhibition was not observed with fairness cream.

DISCUSSION

The experimental results indicate that the topical application of the three personal care products; talc-cum-powder, deospray and fairness cream on the body can restrict the colonization of hostile and invading microorganisms on skin microbiota; a phenomenon referred to as competitive exclusion(Prescott,2008). The environment of the skin also predisposes the skin to selective colonization. But when the skin conditions are changed by the use of topical applications such as creams, powders, lotions, sprays and other cosmetics, the chemicals that are present in the preservatives may alter in the population of skin biota. Though the function of preservative is to maintain the shelf life of a product, they may be absorbed into the inner parts of skin microbiota or sometimes even into the blood without undergoing contamination. This may induce variations on the superficial part of the skin resulting in decrease in the number of microbes. When the effect of a chemical is neutralized or weakened, then again there will be raise in the microbial number. The regeneration of colonies may be either from native microbes or from foreign source. This change in colonization with different microbes will also change the composition of normal microbiota thus leading to the diseases in immune competent cases.

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REFERENCES

1. Fierer N, Hamady M, Lauber CL, Knight R. The influence of sex, handed- ness, and washing on the diversity of hand surface bacteria. Proc. Natl. Acad. Sci. U.S.A., 2008; 105 : 17994–17999.
2. Fredricks DN, Microbial ecology of human skin in health and disease. J. Invest. Dermatol. Symp. Proc. 2001; 6:167–169.
3. Grice EA, Kong HH, Conlan S, Deming CB, Davis J, Young AC, et al. Topo- graphical and temporal diversity of the human skin microbiome. Science. 2009;324: 1190.
4. Roth RR, James WD., Microbial ecology of the skin. Ann. Rev. Microbiol. 1988;42: 441–461.
5. Iwase T, Uehara Y, Shinji H, Tajima A, Seo H, Takada et al., Staphylococcus epidermidis Esp inhibits Staphylococcus aureus biofilm formation and nasal colonization. Nature. 2010;465 : 346–349.
6. Cogen AL, Nizet V , Gallo RL., Skin microbiota: a source of disease or defence? Br. J. Dermatol. 2008;158: 442–455.
7. Heidi H Kong, Skin microbiome: genomics-based insights into the diversity and role of skin microbes.Trends in Molecular Medicine.2011;17:320-327.
8. Fierer N, Christian L, Lauber, Nick Zhou, Daniel McDonald, Elizabeth K. Costello et al. Forensic identification using skin bacterial communities. Proc. Natl. Acad. Sci. U.S.A. 2010; 107: 6477–6481.
9. Professor Keith T. Holland Richard A. Bojar. Cosmetics American Journal of Clinical Dermatology . 2002; 3: 445-449.
10. Leyden JJ Marples RR Mills OH Jr . Gram-negative folliculitis - a complication of antibiotic therapy in acne vulgaris. Br J Dermatol. 1973 88: 533-8.
11. Miller YW, Eady EA, Lacey RW, Cove J H, Joanes DN & Cunliffe W J. et al. Sequential antibiotic therapy for acne promotes the carriage of resident staphylococci on the skin of contacts. J AntimicrobChemother. 1996; 38: 829-37.
12. Russell AD. Mechanisms of bacterial insusceptibility to biocides. Am J Infect Control 2001; 29: 259-61
13. Lalitha Ch, Prasada Rao PVV. Impact of cosmetic blends on Skin Microbiota Anaplastology an open access journal .2012;1 :37.
14. Lansing Prescott, John P.Harley , Donald A.Klein. Normal Microbiota and Nonspecific Host Resistance.Kevin T Kane, James M Smith, Terrance Stanton. Microbiology 5th ed. New York: Von Hoffman Press;2002. p.701.
15. Davin-Regli A, Chollet R, Bredin J, Chevalier J, Lepine F, Pagès JM. Enterobacter gergoviae and the prevalence of efflux in parabens resistance. J Antimicrob Chemother. 2006; 57(4):757-60.
16. Ohalete CN, Okafor CV, Uwaezuoke JC, Nwachukwu MI, Dozie INS, Nwaehiri VL. Antimicrobials effects of deodorants on skin flora. World Journal of Pharmacy and Pharmaceuticals Sciences;1(3):1133-1146.
17. Russel AD.Pseudomonas aeruginosa is highly resistant to triclosan. Whither triclosan. J.Antimicrob. Chemother. 2004; 53(5):693-695.
18. James G Cuppuccino, Natalie Sharman. Biochemical activities of microorganisms. A laboratory manual of Microbiology. Addison Wesley 1999; 4th ed: 129-183. species identification of unknown bacterial cultures 417-421.
19. Watanabe K, Hayano K. Can. J. Microbiol.1993;39:674-680.
20. Edberg SC. US EPA human health assessment: Bacillus subtilis. Unpublished U.S. Environmental Protection Agency Washington D.C. 1991.
21. Arnesen LPS, Fogerlund A, Granum PE . From soil to gut: Bacillus cereus and its food poisoning toxins. FEMS Microbiol. 2008. 32**:**579-606.
22. Lansing Prescott, John P.Harley , Donald A.Klein. Microbial interactions.Kevin T Kane, James M Smith, Terrance Stanton. Microbiology 7th ed. New York: Von Hoffman Press;2008. p.732.