Original Article

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Microbiology Section

Comparison of Normal Resident Flora on the Face of Medical Students who use and who do not use Cosmetics

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ABSTRACT

Introduction: Normal flora of the skin plays a beneficial role in preventing the pathogenic organisms from colonizing the skin and causing infection. It is possible that the facial cosmetics may cause a change in the normal flora disrupting its protective function.

Aim: To find out the effect of cosmetics, those that are applied on to the face on resident normal flora of the face.

Materials and Methods: This was a cross-sectional study involving medical students aged 19 to 25 years, of whom 42 were regular cosmetic users and another 42 were non cosmetic users. Swabs were taken from the face of the subjects after a face wash with soap and water and eluted in 1 ml sterile peptone water. Tenfold dilutions of the sample were made and 100 μl of the diluted sample was spread over the surface of Trypticase soy agar, Mac Conkey's agar and blood agar. The colonies were counted and also identified. Statistical evaluation was done by Chi-square test using SPSS version 16.

Results: We isolated Coagulase Negative *Staphylococci* (CoNS), *Micrococcus* spp. and methicillin sensitive *S.aureus*, non-fermenting oxidase negative gram negative bacilli and diphtheroids from both the groups. We found that most of the students who were regular cosmetic users 22 (52.38%) yielded negligent growth in comparison with non-cosmetic users (16, 38%). CoNS was less frequently isolated from regular cosmetic users 10 (23.8%) in comparison with those who do not use cosmetics 17 (40.47%).

Conclusion: It is possible that regular use of cosmetics does reduce the normal flora but further studies with larger sample size are required to prove and confirm this finding.

Keywords: Bacterial interference, Microbial flora, Skin

INTRODUCTION

The resident micro-organisms are in a dynamic steadiness with the host tissue and the microbial flora may be considered as an essential component of the normal human skin. The resident microbial flora is valuable in occupying a niche and denying its access to transients, which may be harmful and infectious [1]. It is said that resident normal flora inhibit pathogenic bacteria and further process skin proteins, free fatty acids and sebum present in the sweat and sebaceous glands [2].

On mucous membranes and skin, the resident flora may prevent colonization by pathogens and possible disease through "bacterial interference" [3]. The mechanism of bacterial interference may involve opposition for receptors or binding sites on host cells, competition for nutrients, mutual inhibition by metabolic or toxic products, mutual inhibition by bacteriocins antibiotic materials or other mechanisms. Suppression of the normal flora results incolonization by organisms from the environment or other parts of the body which subsequently behave as opportunists or even pathogens [3].

Some cosmetics like skin peels are known to cause deeper exfoliation of skin resulting in break in barrier effect and thus also cause loss of skin flora. Some cosmetics are known to cause alteration in the pH of the skin surface either by increasing or reducing the normal flora of the skin [4]. Preservatives in the product may remain active on the skin and with continued use of the product the resident microbial flora is altered. The risk for this event is dependent on the residual activity of the preservatives in the skin environment [1,4]. Although, the risks associated are low, it is necessary to monitor these changes in normal flora to predict outcomes, whether good, neutral or of concern [1,4]. So in the present study we make an attempt to find out the effect of cosmetics, only those that are applied on to the face like face powders and creams on resident normal flora of the face.

MATERIALS AND METHODS

It is a cross-sectional study involving medical students studying in Kasturba Medical College, Mangaluru, India, aged between 19 to 25 years conducted in the year 2014 for the duration of two months (June and July). The subjects were divided into two groups, those who do not use cosmetics and those who have been using cosmetics every day for at least past one year. Those who do not belong to the above mentioned age group and those who occasionally use cosmetics were excluded from the study.

Assuming 30% reduction of flora amongst those who use cosmetics regularly, as compared to those who do not use, 80% power, 5% alpha error and 10% non response error the sample size comes to 42 in each group and hence the total sample size comes to 84 using the formula:

$n=(Za^2\ pq)/E^2$

The institutional ethics committee clearance has been obtained for the study. The subjects under study were divided into two categories, those who regularly use cosmetics every day at least for the past one year, and those who do not use any cosmetics. Only the cosmetics usually applied on the face like powders, creams and foundations were considered. After obtaining informed consent, the subjects were made to wash their face with soap and water to remove the superficial environmental flora as no amount of scrubbing and washing removes the resident flora. A sterile cotton swab was dipped in 1 ml of peptone water and both the cheeks were swabbed covering an area approximately 1x1 inches and the swab material was eluted into the vials containing peptone water.

Tenfold dilutions of the sample were made and 100 µl of the diluted sample was spread over the surface of Trypticase soy agar, Mac Conkey's agar and blood agar [5]. After overnight incubation at 37°C, the colonies on Trypticase soy agar were counted and multiplied by the dilution factor [5]. The colonies on blood agar and Mac Conkey's agar were identified by standard methods [6]. Methicillin resistance in *S.aureus* was determined by using 30 µg cefoxitin disc as per CLSI guildlines [7]. *S.aureus* with a zone size of ≤21 mm was considered as MRSA. Those students who yielded $\leq 10x10^3$ CFU/ml of peptone water were considered as negligent or no growth; who yielded $\geq 50x10^3$ CFU/ml of peptone water were considered as heavy growth and those who yielded $> 10x10^3$ and $<50x10^3$ CFU/ml of peptone water were considered as moderate growth.

STATISTICAL ANALYSIS

Statistical analysis was done using SPSS version 16 and analyzed. Chi-Square test was used for the comparison across the groups and $p<0.05$ was considered as statistically significant.

RESULTS

Out of the 84 medical students, 16 were male and 68 were female students. There was no correlation between gender and the normal microbial flora population of the facial skin [Table/Fig-1].

We found samples from both groups yielded mostly gram positive cocci (39, 46.42%) followed by gram negative bacilli (6, 7.1%) and gram positive bacilli (5, 5.9%) [Table/Fig-2].

Thirty eight samples yielded negligible growth. It was found that most of the students who were regular cosmetic users 22 (52.38%) yielded negligible growth in comparison with non-cosmetic users 16 (38%), but this difference was not statistically significant (p=0.188).

All samples whether those of cosmetic users or non cosmetic users yielded the same type of flora. The gram positive cocci that formed the facial skin flora consisted of CoNS 27 (32.14%), *Micrococcus* spp.14 (16.7%) and *S.aureus* 8 (9.5%). All the *S.aureus* isolated were MSSA (Methicillin sensitive *S.aureus*). CoNS was less frequently isolated from regular cosmetic users 10 (23.8%) in comparison with those who do not use cosmetics 17 (40.47%) and the difference was statistically significant (p=0.053) [Table/Fig-2].

Bacteria Isolated	Gender		p-value*
	Female (n=68) (%)	Male (n=16) (%)	
Negligible growth**	32 (47)	6 (37.5)	0.489
Gram positive cocci	30 (44.1)	9 (56.2)	0.381
Coagulase negative Staphylococci	20 (29.41)	7 (43.75)	0.269
S.aureus	7 (10.29)	1 (6.25)	0.620
Micrococcus	13 (19.1)	1 (6.25)	0.214
Gram negative bacilli (non-fermenter)	6 (8.8)	0	0.218
Corynebacterium spp	4 (5.8)	1 (6.25)	0.955

[Table/Fig-1]: Correlation between the number of students showing growth of the various bacteria and their gender.
* There was no correlation between gender and the normal facial flora.
** Only the organisms which yielded heavy or moderate growth were identified.

Bacteria isolated	Cosmetic usage		p-value
	Regular (n=42) (%)	Non cosmetic user (n=42) (%)	
Negligible growth**	22 (52.38)	16 (38)	0.188
Gram positive cocci	17 (40.47)	22 (52.38)	0.133
Coagulase negative Staphylococci	10 (23.8)	17 (40.47)	0.053*
S.aureus	6 (14.28)	2 (4.76)	0.178
Micrococcus	7 (16.66)	7 (16.66)	0.845
Gram negative bacilli (non- fermenter)	4 (9.52)	2 (4.76)	0.467
Corynebacterium spp.	3 (7.14)	2 (4.76)	0.725

[Table/Fig-2]: Correlation between the number of students showing growth of the various bacteria and use of cosmetics on the face.
*Coagulase negative staphylococci were isolated more frequently from facial flora of people who do not use cosmetics in comparison with those who use cosmetics regularly.
**Only the organisms which yielded heavy or moderate growth were identified.

All the gram negative bacilli isolated were of the same type and non-fermenters 6 (7.1%). There was no statistical correlation between the cosmetic usage and gram negative bacilli population of the facial flora. All the gram positive bacilli isolated were *Corynebacterium* spp. 5 (5.9%). There was no correlation between the cosmetic usage and *Corynebacterium* spp. population of the facial normal flora [Table/Fig-2].

The average colony count of those who yielded negligible growth was $5.13x10^3$ CFU/ml; those who yielded moderate growth was $30.23x10^3$ CFU/ml; those who yielded heavy growth was $88.03x10^3$ cfu/ml. Regular cosmetic users yielded an average colony count of $16.47x10^3$ CFU/ml of CoNS and non-cosmetic users yielded an average colony count of $29.97x10^3$ CFU/ml.

DISCUSSION

As our study is on facial flora, we have studied only the aerobic population. Previous studies have looked for the presence of anaerobic flora also as these studies are based on skin flora in general [2]. The predominant resident microbial flora of the skin are aerobic and anaerobic Gram positive bacilli like *Corynebacterium* spp, *Propionibacterium* spp; *Staphylococcus epidermidis*, other CoNS occasionally *S.aureus*, *Viridans streptococcus, Enterococcus* spp and *Peptostreptococcus* spp; Gram-positive aerobic, spore forming bacilli that are ubiquitous in air, water and soil; and Gram-negative coliform bacilli and *Acinetobacter* spp [3]. We also isolated similar organisms. We used "cosmetics" and "normal flora" as key words and searched the internet for related studies and we found very few similar studies, and most of them were review articles. But there are no original research articles on cosmetics usage and reduction of normal flora of the face to the best of our knowledge.

A study showed that using some prebiotics like several plant extracts (e.g., Ginseng or black currant) being used as cosmetics inhibit the inflammation causing bacterium *Propionibacterium acnes*, but do not affect beneficial species like CoNS. After three weeks of treatment, the microbial flora of 91% of the volunteers had been rebalanced in this way. This has been used as treatment for acne [8]. An in vitro study revealed the antibacterial effect of the medicated soaps. They believed that the soaps can bring a reduction in the normal flora of the body [9]. Another in vitro study showed that when some preservatives are used in combination in skin care products/cosmetics they reduce the normal flora and reduce the pH of the skin to recommended levels [10].

The above studies showed that the used of cosmetics or soaps and detergents does cause a significant change in normal flora. As our participants were medical students who are exposed to hospital environment, we expected a lot of methicillin resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas* spp. but we have found mostly CoNS and few cases of *S.aureus* but they were all methicillin sensitive.

A review article highlighted that CoNS which are the predominant normal flora of the skin act as the first line of defense against pathogens by producing bacteriocins, toxic peptides against *S.aureus* but rarely damage the keratinocytes. They play asupplementary protective role by influencing the innate immune response of keratinocytes through Toll-Like Receptor (TLR) signaling [11]. As we isolated CoNS more frequently from facial flora of people who do not use cosmetics in comparison with those who use cosmetics regularly, this point may be of concern as these organisms are considered beneficial as first line of defense against more dangerous pathogens like *S. aureus*.

Further, studies are required wherein the non cosmetic users after checking their normal flora are asked to use cosmetics regularly for a fixed period of time and then we may check for any change in the flora.

LIMITATION

Further studies involving more number of participants are required to confirm the present results.

CONCLUSION

We isolated CoNS more frequently from those who do not use cosmetics rather than regular cosmetic users. So it is possible that regular use of cosmetics does reduce the beneficial normal flora but further studies with larger sample size are required to prove this finding.

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