

## Original Research Article

## Impact of Toners on Face Microbiota: A Pilot Study

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**Abstract:** Consciousness of facial care among all age groups has become a global demand. In this scenario Toner becomes a game-changer adding formula to remove impurities, prepares the skin for next skincare steps, balances the pH levels, and restores moisture which is essential for maintaining a healthy and vibrant complexion. As our face harbours a diverse microflora regularly, such products should be tested for their antimicrobial efficacies to establish a scientific bridge. Hence, our study aimed to evaluate the antimicrobial effects of some popular toners against face isolates. For this study, bacteria were isolated from the face of ten persons and identified partially through cultural, microscopic and biochemical observations. Also the antibiotic sensitivity pattern of those face microflora was studied against different antibiotics. The isolates were resistant towards Chloramphenicol, Ampicillin, Amoxicillin, Penicillin and Cefixime, however they were found sensitive towards Kanamycin, Gentamycin, Ciprofloxacin, Tetracycline, and Azithromycin. For the key work nine numbers of most accepted toners were selected to check their antimicrobial activities against those isolates. Biotique toner showed excellent antimicrobial activity against all the isolates. Hence it is recommended to add toners to every day practices to prevail over microbial accumulation on face leading to various discomforts.

**Keywords:** Face, Toner, facial care, antimicrobial, Biotique, microbiota.

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## INTRODUCTION

Human skin is the largest organ covering the external surface and its main function is to offer external protection against pathogens [1]. The skin forms an ecosystem composed of 1.8 m<sup>2</sup> of various habitats with huge quantity of folds, specialized niches and invaginations to facilitate several groups of microorganisms [2]. Face is the unique aspect of each individual. It is also covered superficially by skin [3]. It is exposed to a multitude of elements such as dust, pollution and a constant stream of immigrant microbes every time [4]. The face recurrently meets dirt, oil, cosmetic residue, and environmental contaminants due to its high exposure. Inadequate cleansing of the face skin can result in inflammation, clogged pores, and the onset of other skin diseases. Hence face cleansing practices are highly essential for healthy skin [5]. Healthy facial skin is everyone's dream. The face can be cleaned using various face care products available in the local market like cleansing milk, cleansing cream, face wash, toners [6]. Such products are soap free, non-irritating, non-allergic, pH balanced and oil controlling which provide additional benefits in terms of removing

sebum, dirt and microorganisms from face [7]. Toner refers to a lotion, tonic or wash designed to cleanse the skin and shrink the appearance of pores. It also moisturizes, protects and refreshes the skin. Toners can be applied to the skin in different ways like, with the help of cotton ball, spraying onto the face or by applying a facial mask covered with toner [8]. It is completely chemical-free and it provides a soothing effect to the skin, as well protect the skin from external elements and also sunburn [9]. Many of the environmental impurities and cosmetic products are not water soluble, hence washing the skin with simple water is not sufficient to remove them. Substances capable of emulsifying them into finer particles should be used for making these fat soluble impurities water soluble. In such circumstances face toners fit best into the picture. They will act as surface active substances that lower the surface tension on the skin and remove dirt, sebum, oil of cosmetic products, microorganisms, and exfoliated corneum cells in an emulsified form [10].

Growing awareness of skin care and protection among the global millennial population drives the market growth of toners. Even the Dermatologists are

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recommending using skin toners to treat a variety of conditions. In addition, the increasing usage of make-up products is advancing the escalation of toner use to an altitude. The impurities and make up products of face may support more microbial lodging if left unclean. As people are using toners regularly to get rid of any microbial infection, it is essential to study the antimicrobial effect of toners. This present study was aimed to evaluate different face toners available in the market in terms of their antimicrobial properties against the flora logged on the skin of human face.

## MATERIALS AND METHODS

The growth media including biochemical reagents, staining agents and antibiotic discs used for this study were procured from Hi-Media, Mumbai. According to market scenario nine different brands of commercially popular face toners were purchased from the local market for the present study *viz.* Himalaya, Oriflame, Biotique cucumber, Dabur, Plum, Lakme, Ayur, Wow, and Mamaearth.

### Isolation of face microbiota

For collection of sample sterile cotton swabs moistened with sterile distilled water were taken and swabbed over the face. The cotton swabs containing sample were inoculated to nutrient agar (NA) plates. After 24 hours of incubation at 37<sup>o</sup> C, the appearances of growth in the form of colonies were taken into account for further studies.

### Bacteriological analysis of the face isolates

The colonies were observed visually and grouped according to their distinguishing characteristics shown on the nutrient plate. One isolated colony from each group was taken to develop the pure culture. After this, the cultures were identified on the basis of their morphological, microscopic, and biochemical characteristics.

### Cultural Characteristics Study

The isolated strains grown on NA plates were subjected to identification by following the diagnostic microbiology based on the standard physical parameters like Colony appearance, shape, margin, elevation, texture, optical density, coloration, odour etc.

### Microscopic Study

The face isolates were Gram stained and visualized under the optical microscope at various magnifications (100X, 400X and 1000X).

### Biochemical Characterization

Different biochemical tests were performed to partially identify the face isolates like Indole test, Methyl red test, Voges-Proskauer test, and Citrate utilization test.

### Antibiotic Susceptibility Test (AST)

The Antibiotic Susceptibility of the face isolates to a multiple number of antibiotics was performed by

Kirby-Bauer disc diffusion method (11) to check their pattern. O/N (10<sup>5</sup> cells/ml) bacterial culture was swabbed on NA plate maintaining 1:100 dilutions. Sterile antibiotic discs of Penicillin, Kanamycin, Gentamycin, Amoxicillin, Azithromycin, Chloramphenicol, Ampicillin, Ciprofloxacin, Cefixime, and Tetracycline were placed on the agar surface. Following incubation the activity were observed in the form of zones of inhibition and the diameter of the zones were measured in mm. The zone sizes were correlated with Kirby Bauer interpretation chart and bacteria were categorized as Resistant (R), Sensitive (S) and intermediately sensitive (IS).

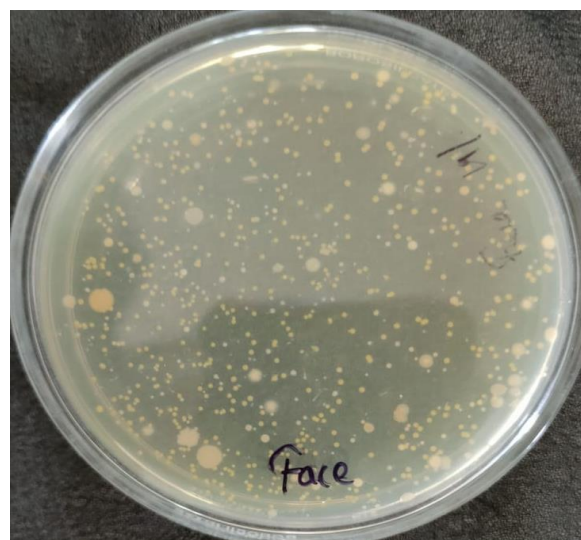
### Assessment of antibacterial efficacy of toner

The antibacterial efficacies of the nine selected face toners were determined qualitatively by well diffusion method. For this purpose, Nutrient agar plates were taken and O/N (10<sup>5</sup> cells/ml) bacterial culture was swabbed maintaining 1:100 dilutions. The toner samples were loaded into 5 mm wells (100 µl in each) cut over the agar surface. After overnight incubation, inhibition was detected by a zone of clearing around the sample spots.

## RESULTS AND DISCUSSION

### Isolation and identification of bacterial strains

Different types of bacterial colonies were obtained from ten different faces (Fig 1). From the mixed culture ten numbers of colonies were selected *viz.* F1, F2, F3, F4, F5, F6, F7, F8, F9, F10 and raised to pure culture for further studies basing upon the distinguished morphology.



**Fig 1: Nutrient Plates showing mixed culture from the face**

The results regarding the colony characteristics and Gram's staining of the face isolates are displayed in Table-1 and the biochemical test result is displayed in Table-2.

**Table-1: Cultural characteristics and Gram’s Staining result**

Isolates	Morphology	Gram’s reaction
F1	Smooth, shiny, medium size, circular, convex, entire margin, Yellow colour, opaque	Gram positive Cocci
F2	Smooth, small size, circular, raised, entire margin, White colour, opaque	Gram positive Cocci
F3	Rough, Irregular margin, Off white colour, Powdery, opaque	Gram positive Rods
F4	Smooth, shiny, medium size, circular, convex, entire margin, Orange colour, opaque	Gram negative Rods
F5	Smooth, shiny, small size, circular, convex, entire margin, white colour, opaque	Gram negative Rods
F6	Smooth, shiny, medium size, flat, entire margin, orange colour	Gram negative Rods
F7	Smooth, shiny, small size, circular, convex, entire margin, white colour, opaque	Gram positive Cocci
F8	Smooth, small size, circular, raised, entire margin, cream colour, semi transparent	Gram negative Rods
F9	Small, white colour, convex, entire margin, smooth, shiny	Gram positive Cocci
F10	Medium size, entire margin, shiny, transparent, off-white colour	Gram negative Rods

**Table-2: Response of face isolates to Biochemical reactions**

Isolates	Indole	Methyl Red	Voges Proskauer	Citrate Utilization	Partial inference
F1	-	-	+	-	<i>Staphylococcus</i>
F2	-	+	-	-	<i>Micrococcus</i>
F3	-	+	+	+	<i>Corynebacterium</i>
F4	+	+	-	-	<i>E.coli</i>
F5	-	+	-	+	<i>Proteus</i>
F6	-	-	+	+	<i>Enterobacter</i>
F7	-	-	+	-	<i>Staphylococcus</i>
F8	-	+	-	+	<i>Proteus</i>
F9	-	+	-	-	<i>Micrococcus</i>
F10	+	+	-	-	<i>E.coli</i>

(- refers to negative and + refers to positive)

The bacteriological analysis of the face isolates could give a partial identification on the basis of their cultural, microscopic and biochemical characteristics. The isolates belong to the genera like *Staphylococcus*,

*Micrococcus*, *E.coli*, *Proteus*, *Enterobacter*, and *Corynebacterium* (12).

**Antibiotic Susceptibility Test**

The antibiotic sensitivity pattern exhibited by the face isolates is given in Table-3.

**Table-3: Antibiotic Susceptibility Test**

Antibiotics	Zones of inhibition (in mm) shown by test isolates									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
<b>Kanamycin</b>	20(S)	18(S)	19(S)	16(IS)	18(S)	19(S)	16(IS)	19(S)	17(IS)	20(S)
<b>Chloramphenicol</b>	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	8(S)	0(R)	0(R)
<b>Ampicillin</b>	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)
<b>Penicillin</b>	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)
<b>Amoxillin</b>	10(R)	6(R)	8(R)	5(R)	10(R)	14(R)	8(R)	11(R)	12(R)	12(R)
<b>Gentamycin</b>	18(S)	19(S)	19(S)	20(S)	18(S)	18(S)	15(S)	19(S)	19(S)	21(S)
<b>Ciprofloxacin</b>	27(S)	26(S)	30(S)	31(S)	28(S)	24(S)	27(S)	33(S)	30(S)	32(S)
<b>Tetracycline</b>	20(S)	21(S)	19(S)	20(S)	21(S)	22(S)	23(S)	22(S)	20(S)	24(S)
<b>Cefixime</b>	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)
<b>Azithromycin</b>	24(S)	23(S)	24(S)	22(S)	24(S)	24(S)	24(S)	24(S)	26(S)	27(S)

(R=Resistant, S=Sensitive, IS= Intermediately sensitive)

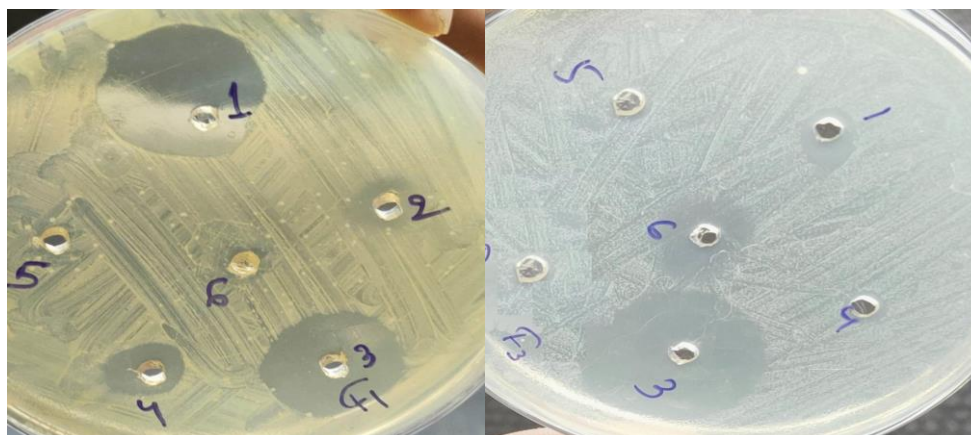
From the antibiotic response pattern of the face isolates it is inferred that all the isolates were resistant towards Chloramphenicol, Ampicillin, Amoxicillin, Penicillin and Cefixime. However they showed sensitivity towards Kanamycin, Gentamycin, Ciprofloxacin, Tetracycline, Azithromycin as interpreted from the Kirby-Bauer Interpretation Chart.

Antibiotic resistance has led to a decreased sensitivity of certain bacterial species of face to

antibiotics [13] due to overuse of such medications. Hence time has come to take necessary measures to limit the development of further antibiotic resistance. Regular care and hygiene of face will add some value definitely.

**Antibacterial efficacies of toners against face isolates**

The antibacterial activities of the toners are depicted in Fig 2 and the measured zones of inhibition around each well are tabulated in Table-4.



**Fig 2: Antibacterial activity exhibited by Toner**

**Table-4: Response of face bacteria to toners**

Toner Brands	Zones of inhibition (in mm)									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
<b>Himalaya</b>	23(S)	0(R)	3(R)	0(R)	6(R)	0(R)	0(R)	3(R)	4(R)	5(R)
<b>Oriflame</b>	6(R)	0(R)	0(R)	0(R)	6(R)	4(R)	0(R)	0(R)	6(R)	7(R)
<b>Biotique</b>	20(S)	26(S)	28(S)	24(S)	27(S)	32(S)	30(S)	24(S)	26(S)	26(S)
<b>Ayur</b>	8(R)	0(R)	0(R)	0(R)	6(R)	4(R)	0(R)	0(R)	0(R)	0(R)
<b>Dabur</b>	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)
<b>Plum</b>	0(R)	0(R)	8(R)	0(R)	10(R)	8(R)	11(R)	7(R)	0(R)	0(R)
<b>Wow</b>	8(R)	0(R)	8(R)	18(S)	8(R)	3(R)	5(R)	6(R)	0(R)	0(R)
<b>Mamaearth</b>	4(R)	6(R)	8(R)	0(R)	0(R)	0(R)	4(R)	10(R)	8(R)	0(R)
<b>Lakme</b>	0(R)	0(R)	3(R)	0(R)	0(R)	11(R)	0(R)	0(R)	0(R)	4(R)

(R=Resistant, S=Sensitive)

From the above assessment it is evident that Biotique Toner is showing remarkable antibacterial activity against all the face isolates. Himalaya is effective against F1 only but it is resistant to other isolates used in this study. As it is an *in vitro* assessment directly against the face isolates, so it may be proposed that the ingredients present in the Biotique Toner are active in inhibiting the face microflora to a greater extent.

The main objective of the present study was to make a qualitative assessment of some common toners against bacteria associated with our face. Addition of such dermocosmetics to our daily skin care routine can improve the skin status to a large degree. Many of the Skincare experts also advise relying on toner for all kinds of skin.

## CONCLUSION

The scientific knowledge about these cosmetic products among people is very little and a multiple of reports on possible effects of the use of toners are circulating in the media. Facial toner is on a whole the in-between skincare step. It should be used after washing the face but before applying moisturizer. During earlier days, toners were used to balance the pH of skin after using an alkaline soap product for cleansing [14]. Subsequent to extensive research by dermatologists and skin care experts now it is believed that toners provide different benefits. They prepare the skin for moisturizers

and serum while getting rid of excess oil and stubborn dirt or makeup leftover on the face after washing. They are used to target a changing array of skin concerns from acne to dryness to aging. So toners are now considered as the most useful cosmeceutical items adding benefits in terms of rehydrating skin, adjusting skin pH, fixing skin pores, easing aggravation, and furthermore antisepsis [15].

Furthermore as our face gets contaminated frequently in this polluted environment, it is necessary to wipe out the colonized microbes to get rid of skin problems. This study therefore has provided evidence to show the efficacy of the use of toner in helping the face to lower microbial population. Hence it is suggested that attention be paid to personal hygiene by using such type of cosmetic products to lead a better social life.

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**Conflict of Interest:** The authors declare no conflict of interest for this publication.

## Statement of informed consent

The Authors are stating that Informed consent was obtained from all the individual participants included in this study.

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