

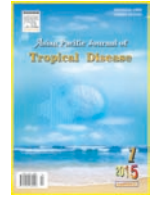
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Demonstration of *in vitro* antibacterial activity of the popular cosmetics items used by the Dhaka locality

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ABSTRACT

Objective: To demonstrate the antibacterial activity of cosmetic products commonly used by the community of Dhaka metropolis.

Methods: A total of 10 categories of cosmetic samples (with a subtotal of 30 brands) were subjected to microbiological analysis through conventional culture and biochemical tests. Agar well diffusion method was used to determine the antibacterial trait in the tested samples which was further confirmed by the minimum inhibitory concentration method.

Results: All samples were found to be populated with bacteria and fungi up to 10^5 CFU/g and 10^3 CFU/g, respectively. Growth of *Staphylococcus* spp., *Pseudomonas* spp. and *Klebsiella* spp. was recorded as well. Conversely, 7 out of 30 items were found to exhibit the *in vitro* antibacterial activity against an array of laboratory test bacterial species including *Staphylococcus* spp., *E. coli*, *Bacillus* spp., *Pseudomonas* spp., *Klebsiella* spp. and *Listeria* spp. Consequently, all the samples showed antibacterial activity below the concentration of 0.46 mg/mL as found in the minimum inhibitory concentration test.

Conclusions: Overall, the presence of huge microbial population in cosmetic products is not acceptable from the point microbiological contamination level. The antibacterial trait of these items, in contrary, may draw an overall public health impact.

1. Introduction

Pharmaceutical finished products especially in the developing countries encounter microbial contamination due to certain discrepancies during manufacturing and packaging stages followed by problems in the storage conditions[1-5]. Cosmetic products, which are not significantly different from those of pharmaceutical medicaments regarding the manufacturing process or storage fashion during sales, may also undergo contamination by a range of microorganisms[6-10]. Indeed, besides the pharmaceutical health care medicinal products, the cosmetic items have also long been

reported to be prone to microbial attack including bacteria and fungi[11-14]. Like the pharmaceutical products or the preservatives, the microbiological quality of the cosmetic items has also been restricted by the acceptable limits of microorganisms (for non-eye area $< 10^3$ CFU/g and for eye area $< 10^2$ CFU/g) recommended by the regulatory bodies including the British Pharmacopeia or the United States Pharmacopeia (USP), Food and Drug Administration (FDA), etc.[6,15,16]. Exceeding the recommended microbial bioburden in the consumer health care, cosmetic products may result in several types of disease complications including scabies, acne, eczema, dyschromia and others upon the topical application[15-19]. Therefore, consistent and reliable microbiological quality monitoring of the available cosmetic items are required to ensure not only the consumer safety but also the overall public health[6,20].

Our recent study regarding the common cosmetic items revealed a huge contamination by the heterotrophic bacteria, fungi, and

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the specific pathogenic bacteria as well[6]. In context of the pharmaceutical medicines, a nearly similar scenario has also been noticed[3-7]. Interestingly, some of the pharmaceutical products have been found to exhibit the antibacterial activity which indeed and to some extent ensure the product safety in the concept of killing harmful bacteria[5-7,21]. These lines of evidence further encouraged us to check for the possible existence of the antibacterial traits in the cosmetic samples. Thus in cohort with the previous work, the present study attempted to: (1) isolate and detect the cosmetics contaminating microorganisms including the specific bacterial pathogens, fungi and actinomycetes; (2) further to demonstrate the *in vitro* antibacterial activity of those samples.

2. Materials and methods

2.1. Sampling and sample processing

Thirty brands of 10 categories of cosmetics, *i.e.*, 3 in powder formulations, 3 deodorant roll-on, 3 lipsticks, 3 herbal formulations of mehedi (*Lawsonia inermis*), 3 hair remover creams, 4 hair gel, 4 sunscreen lotions, 3 lip glosses, 2 moisturizer creams and 2 anti-ageing cream samples were used in this study within a time frame of November 2013 to March 2014. Samples (all in the form of finished products) were randomly collected from different health-care stationary shops in Dhaka city. Dates of manufacturing and expiry were checked prior to microbiological tests. For the estimation of bacterial and fungal load, each of the cosmetic products were shaken thoroughly and samples were then taken from the surface of the respective products, and were well mixed with buffer peptone water (in 1:10 ratio). Serial dilutions were then consecutively prepared up to 10^{-5} [6].

2.2. Enumeration of total viable bacteria and fungi

A total of 0.1 mL of each suspension from the dilution 10^{-2} was introduced into the nutrient agar plates and Sabouraud dextrose agar plates by means of spreading in order to isolate and quantify the total viable bacterial count and fungi, respectively[6,7,22]. The nutrient agar plates were incubated at 37 °C for 18 to 24 h and the Sabouraud dextrose agar plates were incubated at 25 °C for 48 to 72 h, respectively.

2.3. Enumeration of specific pathogens and actinomycetes

From the dilution of 10^{-2} of each sample, 0.1 mL of suspension was spread onto MacConkey agar, mannitol salt agar, cetrimide agar, phenol red egg yolk polymyxin (mannitol yolk polymyxin) agar base and Bennet agar (composed of yeast and beef extracts, casein enzyme hydrolysate and dextrose and supplemented with nystatin) media for the enumeration of *Escherichia coli* (*E. coli*), *Staphylococcus* spp., *Pseudomonas* spp., *Bacillus* spp. and *Actinomycetes*, consecutively. All the plates were incubated at 37 °C

for 24 h. Appearance of the typical colonies, such as pink colonies on MacConkey agar, yellow colonies on mannitol salt agar, colonies with greenish pigmentation on cetrimide agar, colonies with clear zone on mannitol yolk polymyxin agar and colorless colonies on Bennet agar, was analytical for the growth of *E. coli* or *Klebsiella* spp., *Staphylococcus* spp., *Pseudomonas* spp., *Bacillus* spp. and actinomycetes, consecutively[6,7,23]. Finally, the confirmative biochemical tests were conducted to ensure the identity of the isolates[6,22,24,25].

2.4. Determination of antibacterial activity of the cosmetic samples

The investigation of the antibacterial activity of the cosmetic samples was primarily performed by using the agar well diffusion method[26,27]. Briefly, cosmetic blends were used directly on the Mueller-Hinton agar (MHA) media. At first, the bacterial suspensions of the laboratory strains (*Pseudomonas* spp., *Listeria* spp., *Bacillus* spp., *Vibrio* spp., *Salmonella* spp., *Klebsiella* spp., *Staphylococcus aureus*, *E. coli*) with the equivalent turbidity standard of McFarland (0.5) were introduced evenly over the MHA media separately using the sterile cotton swabs. Wells were spanned across the MHA by a sterile cork borer[1,28-30]. From each of the crude cosmetic blends, an aliquot of 100 μ L was introduced into the wells. Normal saline was applied as the negative control while 10 μ g of the antibiotic disc of gentamicin was used as the positive control. Presence of clear zone(s) around the samples was indicative of the antibacterial potential of the cosmetic samples employed.

2.5. Determination of minimal inhibitory concentration (MIC)

The MIC was determined to observe the lowest concentration of the samples in which the bacterial load was inhibited[31]. Two fold serial broth dilution methods were applied to determine the MIC of samples according to the guidelines established by Clinical and Standard Laboratory Institute[32]. The suspension of different test organisms was prepared to amend their turbidity using 0.5 McFarland standard, and the Mueller-Hinton broth (Oxoid Ltd, England) was prepared in sterile test tube with 100 μ L of the test bacterial inoculum. Then different concentrations of cosmetics were introduced into the inoculum containing broth and all the tested tubes were incubated at 37 °C for 24 h. The smallest amount of samples which could retard the multiplication of the tested bacteria (as indicated by measuring the zero density) was regarded as MIC.

3. Results

3.1. Prevalence of microorganisms in the cosmetic samples studied

In the present study, of the 10 categories of samples studied, all samples were found to exhibit elevated load of total viable bacteria

Table 1
Prevalence of pathogenic microorganisms in different types of cosmetics (CFU/g).

Samples		Total viable bacterial count	Total fungal count	<i>E. coli</i>	<i>Klebsiella</i> spp.	<i>Staphylococcus</i> spp.	<i>Pseudomonas</i> spp.	<i>Actinomycetes</i>
Powder	Magic prickly-heat	2.0×10^4	6.0×10^4	0	0	2.0×10^2	2.0×10^1	0
	MariTelcom powder	3.0×10^5	5.0×10^4	0	0	3.0×10^2	1.0×10^2	0
	Vatiny face powder	2.1×10^4	4.0×10^3	0	0	1.4×10^3	3.0×10^2	0
Deo roll on	Fa	1.1×10^4	4.0×10^3	0	0	7.0×10^2	2.0×10^2	0
	She	3.0×10^4	2.0×10^4	0	0	3.0×10^3	1.0×10^2	0
	Rexona men	3.0×10^5	2.2×10^4	0	0	5.0×10^2	2.0×10^2	0
Lipstics	La femme	5.0×10^5	2.0×10^4	0	0	2.0×10^3	0	0
	Heng fang	3.5×10^5	5.7×10^3	0	0	2.4×10^2	0	0
	Loreal	2.6×10^4	3.0×10^3	0	0	6.7×10^1	0	0
Mehedi	Mumtaz gold no-1	1.3×10^5	3.1×10^4	0	0	2.0×10^2	4.0×10^2	0
	Ligion active gold	2.0×10^4	4.1×10^3	0	0	3.1×10^2	2.4×10^2	0
	Smart active	1.4×10^5	4.0×10^2	0	0	0	0	0
Hair remover cream	Nair hair remover	1.5×10^4	2.5×10^4	0	0	0	0	0
	Fem anti darkening	2.0×10^5	2.0×10^4	0	0	0	2.0×10^1	0
	Cosmo silky	3.1×10^5	3.1×10^4	0	1.5×10^5	3.0×10^3	4.0×10^2	0
Hair gel	Extrme style	1.5×10^5	7.0×10^3	0	0	2.0×10^2	2.0×10^2	0
	Set in style	1.0×10^5	2.0×10^2	0	0	1.5×10^3	3.0×10^1	0
	Pop popular	2.0×10^5	1.0×10^3	0	0	3.0×10^2	2.0×10^2	0
	Gillette	3.1×10^4	1.1×10^1	0	0	2.1×10^1	3.0×10^2	0
Sunscreen lotion	Neutrogena	1.0×10^5	2.0×10^2	0	0	1.0×10^2	1.5×10^1	0
	Loreal Paris	1.0×10^5	3.0×10^3	0	0	2.0×10^3	1.4×10^1	0
	Somis Ayurvedic	1.5×10^5	2.0×10^3	0	0	1.4×10^2	2.0×10^2	0
	Boro Plus	1.3×10^5	4.0×10^3	0	0	3.0×10^3	1.2×10^1	0
Lip gloss	Ludanmei	1.5×10^5	2.0×10^3	0	0	2.5×10^3	2.0×10^2	0
	Drevn	1.1×10^5	1.0×10^2	0	0	3.0×10^2	2.0×10^1	0
	Clipon	1.2×10^5	5.0×10^3	0	0	2.0×10^3	3.0×10^1	0
Moisturizer cream	Clean and Clear	4.0×10^5	1.2×10^3	0	1.0×10^3	1.4×10^3	1.3×10^2	0
	Olay	3.0×10^5	1.0×10^2	0	0	1.0×10^3	1.0×10^1	0
	Antiageing Cream							
	Pond's	3.0×10^5	2.0×10^2	0	2.0×10^3	2.5×10^3	2.0×10^2	0
Olay	4.0×10^5	1.0×10^2	0	0	0	1.0×10^1	0	

USP or FDA limit of aerobic bacteria $< 10^3$ CFU/g for products of non-eye area. Three samples from the same lot of each cosmetic product were used. The results were reproducible. Only one representative data has been shown.

Table 2
Confirmative biochemical tests for the isolates.

Assumed organism	Triple sugar iron test			H^2 sreaction	Indole test	Methyl red	Voges-Proskauer test	Citrate test	Motility test	Oxidase test
	Slant	Butt	Gas							
<i>Klebsiella</i> spp.	Y	Y	-	-	-	-	-	-	-	+
<i>Pseudomonas</i> spp.	Y	Y	-	-	-	-	-	+	-	-
<i>Staphylococcus</i> spp.	Y	R	+	+	-	+	-	+	+	-

Y: Yellow (acid); R: Red (alkaline).

up to 10^5 CFU/g which eventually exceeded the USP or FDA limit of $< 10^3$ CFU/g for non-eye area (Table 1). The fungal load was estimated within the range of 10^1 – 10^3 CFU/g (Table 1). Among the pathogenic bacteria (Tables 1 and 2), *Staphylococcus* spp. were found in almost all samples (in an average of 10^2 CFU/g) except Smart active mehedi, Nair hair remover, Fem anti-darkening and Olay Anti-ageing cream. *E. coli* and *Actinomycetes* were found to be totally absent in all samples. *Klebsiella* spp. were found to be present in Cosmo silky hair remover cream, clean and clear moisturizer cream and Pond's anti-aging cream. *Pseudomonas*

spp. was found to be present in almost samples except lipstics (La femme, Heng fang and Loreal), Smart active mehedi and Nair hair remover cream (Table 1).

3.2. In vitro antibacterial activity of the cosmetic samples

The *in vitro* antibacterial activity of several cosmetics items including the Magic prickly heat powder, Smart active Mehedi, Fa deo roll on, Neutrogena and Loreal Paris sun screen cream, Olay moisturizer cream, Pond's and Olay antiageing cream were noticed

Table 3

Antimicrobial activity of the cosmetics (measured by agar well diffusion method)

Samples		Zone size of inhibition (mm)							
		<i>E. coli</i>	<i>Bacillus</i>	<i>Staphylococcus</i>	<i>Vibrio</i>	<i>Salmonella</i>	<i>Pseudomonas</i>	<i>Klebsiella</i>	<i>Listeria</i>
Powder	Magic prickly-heat	13 mm	11 mm	10 mm	-	-	14 mm	16 mm	15 mm
	MarilTelcom powder	-	-	-	-	-	-	-	-
	Vatiny face powder	-	-	-	-	-	-	-	-
Deo roll on	Fa	26 mm	25 mm	28 mm	-	-	23 mm	-	-
	She	-	-	-	-	-	-	-	-
	Rexona men	-	-	-	-	-	-	-	-
Lipstics	La femme	-	-	-	-	-	-	-	-
	Heng fang	-	-	-	-	-	-	-	-
	Loreal	-	-	-	-	-	-	-	-
Mehedi	Mumtaz gold no-1	12 mm	15 mm	13 mm	-	14 mm	-	-	-
	Ligionactive gold	-	-	-	-	-	-	-	-
	Smart active	-	-	-	-	-	-	-	-
Hair remover cream	Nair hair remover	-	-	-	-	-	-	-	-
	Fem anti darkening	-	-	-	-	-	-	-	-
	Cosmo silky	-	-	-	-	-	-	-	-
Hair gel	Extrme style	-	-	-	-	-	-	-	-
	Set in style	-	-	-	-	-	-	-	-
	Pop popular	-	-	-	-	-	-	-	-
	Gillette	-	-	-	-	-	-	-	-
Sunscreen lotion	Neutrogena	13 mm	-	-	-	-	15 mm	-	-
	Loreal Paris	12 mm	-	-	-	-	13 mm	-	-
	Somis Ayurvedic	-	-	-	-	-	-	-	-
	Boro Plus	-	-	-	-	-	-	-	-
Lip gloss	Ludanmei	-	-	-	-	-	-	-	-
	Drevn	-	-	-	-	-	-	-	-
	Clipon	-	-	-	-	-	-	-	-
Moisturizer cream	Clean and Clear	-	-	-	-	-	-	-	-
	Olay	15 mm	15 mm	-	-	-	-	-	-
Antiageing cream	Pond's	12 mm	14 mm	-	-	-	-	-	-
	Olay	13 mm	13 mm	-	-	-	-	-	-

Table 4

MIC of the cosmetics (measured by broth microdilution method).

Samples		Concentrations of the cosmetics (mg/mL)							
		<i>E. coli</i>	<i>Bacillus</i>	<i>Staphylococcus</i>	<i>Vibrio</i>	<i>Salmonella</i>	<i>Pseudomonas</i>	<i>Klebsiella</i>	<i>Listeria</i>
Powder	Magic prickly-heat	0.11	0.11	0.23	0.23	0.23	0.23	0.11	0.11
	Maril telcom powder	0.23	0.23	0.23	0.23	0.46	0.23	0.23	0.46
	Vatiny face powder	0.11	0.46	0.23	0.23	0.23	0.46	0.23	0.46
Deo roll on	Fa	0.11	0.11	0.23	0.23	0.23	0.11	0.11	0.23
	She	0.23	0.11	0.23	0.46	0.11	0.46	0.23	0.46
	Rexona men	0.11	0.23	0.23	0.23	0.46	0.22	0.23	0.23
Lipstics	La femme	0.11	0.23	0.23	0.46	0.46	0.46	0.23	0.46
	Heng fang	0.11	0.46	0.23	0.46	0.22	0.23	0.23	0.46
	Loreal	0.23	0.23	0.23	0.22	0.23	0.23	0.23	0.46
Mehedi	Mumtaz gold no-1	0.11	0.11	0.11	0.11	0.11	0.23	0.11	0.11
	Ligionactive gold	0.11	0.11	0.11	0.23	0.46	0.23	0.23	0.46
	Smart active	0.23	0.23	0.23	0.23	0.11	0.46	0.23	0.23
Hair remover cream	Nair hair remover	0.23	0.46	0.11	0.46	0.23	0.11	0.11	0.23
	Fem anti darkening	0.23	0.46	0.11	0.23	0.23	0.23	0.11	0.46
	Cosmo silky	0.23	0.46	0.23	0.46	0.11	0.46	0.23	0.23
Hair gel	Extrme style	0.23	0.46	0.46	0.46	0.46	0.46	0.46	0.46
	Set in style	0.46	0.46	0.23	0.46	0.23	0.46	0.23	0.46
	Pop popular	0.23	0.46	0.23	0.23	0.46	0.46	0.23	0.46
	Gillette	0.23	0.46	0.23	0.46	0.46	0.23	0.23	0.46
Sunscreen lotion	Neutrogena	0.11	0.46	0.23	0.23	0.46	0.23	0.23	0.46
	Loreal Paris	0.11	0.46	0.23	0.23	0.46	0.11	0.23	0.46
	Somis Ayurvedic	0.11	0.23	0.23	0.23	0.23	0.23	0.23	0.46
	Boro Plus	0.11	0.23	0.23	0.23	0.23	0.23	0.23	0.23
Lip gloss	Ludanmei	0.23	0.46	0.23	0.11	0.23	0.23	0.11	0.23
	Drevn	0.11	0.23	0.11	0.23	0.23	0.11	0.11	0.23
	Clipon	0.11	0.46	0.23	0.11	0.46	0.11	0.11	0.46
Moisturizer cream	Clean and Clear	0.23	0.23	0.11	0.23	0.46	0.11	0.23	0.23
	Olay	0.23	0.23	0.23	0.46	0.23	0.46	0.46	0.46
Antiageing cream	Pond's	0.11	0.11	0.46	0.46	0.23	0.46	0.23	0.46
	Olay	0.11	0.11	0.11	0.23	0.23	0.46	0.11	0.23

in the present study (Table 3). Magic prickly heat powder was found to inhibit the growth of *Staphylococcus* spp., *E. coli*, *Bacillus* spp., *Pseudomonas* spp., *Klebsiella* spp., and *Listeria* spp. Smart active Mehedi effectively reduced the growth of *E. coli*, *Bacillus* spp., *Pseudomonas* spp. and *Salmonella* spp. Fa deo roll on showed antibacterial activity against *E. coli*, *Bacillus* spp., *Pseudomonas* spp. and *Staphylococcus* spp. Neutrogena and Loreal Paris sun screen cream were found to inhibit the growth of *E. coli* and *Pseudomonas* spp. Olay moisturizer cream, Ponds and Olay anti-ageing cream were observed to possess the antibacterial activity against *E. coli* and *Bacillus* spp. (Table 3). Furthermore, the presence of antibacterial properties of cosmetics was confirmed through determining the MIC levels of the samples. All the samples were found to be highly effective against tested bacteria and showed their MIC at 0.11 mg/mL, 0.23 mg/mL and 0.46 mg/mL. Most of the samples exhibited their antibacterial traits at 44 mg/mL against *Listeria* spp. among all tested bacteria (Table 4).

4. Discussion

Presence of huge array of contamination in cosmetic products was found in our recent studies in Bangladesh, and in the other studies on topical. And other pharmaceutical products were urgent need to conduct in the present study[2-6]. Being in the non-sterile categories, indeed, the cosmetic items are unlikely to be attacked by microorganisms which has also been supported by a number of earlier evidences[6,9,33,34]. As demonstrated in several reports, the magnitude of microbial contamination principally depends on the germ-infested conduct of the bulk ingredients during product manufacturing accompanied with inadequate in-process quality inspection, followed by untailored storage state and distribution into the market without the appropriate quality assurance of the finished products[16,35-37].

In consistent with the results delivered from few microbiological studies on cosmetics, present investigation also revealed a huge quantity of heterotrophic bacteria exceeding the recommended limit[9,12,38]. Specific bacterial presence, as well as the fungal proliferation within the samples was not negligible in terms of product quality. The findings of the present study were indeed in harmony with that of the previous study conducted by Das *et al.*, whereby huge load of bacterial pathogens was observed in the local samples' studies[6]. Presence of comparatively higher load of pathogens in present study and the study carried out by Das *et al.* together revealed the public health risk upon usage of the commonly available cosmetic products used in Bangladesh. Nutrient content of the products such as lipids, polysaccharides, alcohol *etc.*, and storage conditions such as availability of O₂, inactivity of preservatives, poor handling *etc.* might be the possible cause of contamination in the cosmetic products[6,34,39-41]. However, further chemical examination would unravel the mechanistic insights behind the microbial contamination of the cosmetics products. Nevertheless, an important

facet of the current investigation projected from the findings of the antibacterial activity of the tested cosmetic samples which were of significant clinical concern.

The current investigation revealed a huge extent of microbial contamination exceeding USP or FDA limit as found previously which actually extended our concern on the uses of these products in public health. Presence of antibacterial activity in a numbers of products might ensure some extent of product safety but their significance on eliminating contaminating bacteria was remained to be investigated. However, stringent regulatory actions on the microbiological quality control along with the personal hygienic improvement during formulation, handling and storage of the products would be effective for the better management of the overall public health situation.

Conflict of interest statement

We declare that we have no conflict of interest.

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