

Fungal Profiles Isolated from Open and Used Cosmetic Products Collected from Different Localities in Saudi Arabia

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Abstract

A total of seventy five samples of cosmetic products open and used for varying periods of time were collected from different localities in Saudi Arabia. These samples were analyzed for the presence of mesophilic and thermophilic fungi. Six genera and 13 species were isolated at 28°C. Eleven genera, 24 species as well as two species varieties were encountered at 45°C (thermophilic fungi). *Aspergillus* was the most common genus while *A. terreus* was the most common species at 28°C and 45°C. The highest total count of fungi was obtained from samples of lip cosmetic products, while the lowest total counts were detected in eye cosmetic products. The antifungal activity was studied for seven randomly selected commercial drugs against five species of isolated saprophytic fungi. Results indicated that Dermocart skin cream was largely effective against all tested fungal isolates.

Key Words: Used cosmetic, Fungi, Isolated, Mesophilic, Thermophilic.

Introduction

Eye liner, eye shadow, eye pencils, etc are meant to make the eyes more attractive and alluring. The majority of eye cosmetics are safe when used properly; however, there are some general Federal Food and Drug Administration (FDA) Guide Lines when purchasing and using eye cosmetics. But misuse and old cosmetics can harbor dangerous bacteria and fungi. There have been rare instances of women being temporarily blinded because of bacteria and/or fungal infection eye make up A slight scratch of the cornea can result in serious eye infection (Klotz *et al.*, 2000 and Belanger, 2007).

The ability of microorganisms to grow and reproduce in cosmetics products has been known for many years (Jasnow and Smith, 1975; Mislivec *et al.*, 1993 and Fujita *et al.*, 2005).

Bacteria and fungi can get to cosmetics and bodycare products in several ways (pat Thomas, 2008). The microbiological quality of finished cosmetics, particularly dry or powdered products, depends largely on the quality of raw materials used in its manufacture (Jasnow and Smith, 1975). For instance, a color additive to every lot can be

expected to contain gram-negative bacteria (Bonorden, 1973). Fungi may also be present and can cause spoilage problems in certain finished products (Berdick, 1973). Mislivec *et al.* (1993) studied samples of shared- use cosmetics that were analyzed for the presence of viable bacteria and fungi. Fungi were present in 10.4% of samples and pathogenic fungi were identified in 3.9%. Fujita *et al.* (2005). Presented rapid and accurate procedures for identifications of microorganisms contaminating cosmetic products based on identification of nucleotide sequences of their respective ribosomal RNA.

Preservatives are customarily used to control cosmetics contamination. For example Euxyl K 135, is equally effective against bacteria, yeast and filamentous fungi (Schülke and Mayr, 1993). Also, kojic acid is commonly used in cosmetic products for its effect on melanocytes in lightening pigments to retard spoilage by various species of *Aspergillus* and *Penicillium* (Chariene De Haven, 2007). Likewise, cosmetic powders would inherently possess antibacterial and antifungal effect, in which a base powder zinc oxide and/or zinc basic carbonate and alkali metal salt

are combined (Yokoyama, 2005).

In the present study samples of commercial cosmetics that were open and used for varying periods of time were collected from different localities in Saudi Arabia and analyzed for the presence of fungi at 28°C and at 45°C and studying the anti-fungal activity of randomly selected drugs against isolated fungal species.

Material and Methods

Samples collection

Seventy five samples of the cosmetics (25 samples from each eye, face and lip products) which were open and used for varying periods of time were collected from different localities in Saudi Arabia (2007) and analyzed for the presence of fungi. These samples of cosmetic products included for eye (eye shadows, liquid mascara, liquid eye liners) for lip (lip sticks, lip glosses and rouges), for face (facial blushers, foundations and face powdered cosmetic).

Each of the tested samples was swabbed with sterile cotton swabs, from the approximately 1 sq. in area of the surface of each solid cosmetic swabbed, as for liquid or semi solid cosmetics swabs were dipped into the containers as in (Mislivec *et al.*, 1993), but with some modification. Each swab was used to inoculate one plate of potato dextrose agar medium (PDA) supplemented with 40 ppm chlortetracycline hydrochloride), 6 plates of the same medium were used for each sample. The swab surface was rotated approximately with the soluble medium in the plates before solidified for 10 min. 3 plates for each sample were incubated at 28°C±2°C for seven days and the other 3 plates were incubated at 45°C±2°C for 7 days for detection of thermophilic and or thermotolerant fungi. The developing fungi were identified and counted.

Antifungal activity test

The disc method was used to measure the antimicrobial activity as described by (Sleght and Timburg, 1981). Test fungal species of old culture were streaking each on the surface of PDA medium plate (15 ml of PDA), and after 4 min of inoculation an appropriate number of small sterile disks of filter paper (Whatman No. 3 filter paper, 0.5 cm diameter), were dipped in several antibiotics that were used of 0.5 ml L/disk for each drugs was added for placing on the surface of inoculated medium, the plates were incubated at 28°C for four days. The diameter of the zone was measured in mm. All of the tests were conducted in triplicates.

Saprophytic fungi: fungal strains used in this study were *Aspergillus flavus*, *A. fumigatus*, *A. terreus*, *A. versicolor* and *Penicillium chrysogenum*. Pure cultures of local isolates of these fungi were obtained from the same cosmetic samples that were studied.

Identification of microfungi

Identification of the fungal isolates were performed according to the Raper and Thom (1949), Raper and Fennell (1965), Barnett & Hunter (1972), Ellis (1971), Gilman (1957) and Moubasher (1993).

Results

Numbers of colonies and isolates for individual species illustrated in the Tables (1 and 2). Plate counts of zero were found for 60% of the samples incubated at 28°C and 20% of samples incubated at 45°C.

Occurrence of mesophilic fungi at 28±2°C. The 73 fungal isolates identified, representing 6 genera and 13 species (Table 1). The results of Table (1) reveal that 6 genera, *Aspergillus*, *Penicillium*, *Chaetomium*, *Cladosporium*, *Rhizopus* and *Phoma* were isolated at 28°C. The highest total counts of fungi (35) were obtained from the samples of Lip cosmetic products followed by (31) were estimated from samples of face cosmetic products, while the lowest total counts of fungi (7) obtained from the samples of eye cosmetic products (3 and 4 eye shadow & liquid mascara, respectively). But liquid eye liners not contaminated with fungi. Although the richest samples in the population of genera and species were isolated from lip and face cosmetic products, while the poorest samples were isolated from eye products (Table 1).

Six species of *Aspergillus* were collected contributing 71.2% of total fungi. *A. terreus* was the most frequent species that were isolated. It constituted 24.7% of total fungi. *A. flavus* and *A. niger* were the second and the third most frequent species respectively comprising (13.7% and 12.3% of total fungi, respectively). The other remaining species of *Aspergillus* were of moderate or low frequencies Table (1).

Phoma leveillei came second after *Aspergillus* constituting 8.2% of total fungi. *Penicillium* was the third, it contributed 6.8% of total fungi. It was represented by two species, *P. chrysogenum* and *P. citrinum* comprising 4.1% and 2.7% of total fungi respectively.

Chaetomium globosum and two species of *Rhizopus*

Table 1. Total counts and percentage of fungi isolated on PDA* medium at 28°C from cosmetic of eye, face and lip-products.

Genera and species of fungi	Eye		Face			Lip	Total count	% of T.C.
	Sh	M	C	P	F			
<i>Aspergillus</i>	3	3	10	4	5	27	52	71.2
<i>A. flavus</i> Link			2	2		6	10	13.7
<i>A. fumigatus</i> Fresen			2			4	6	8.2
<i>A. niger</i> Tiegh			3	2	2	2	9	12.3
<i>A. sydawi</i> (Bain. & Sart.) Thom & Church						2	2	2.7
<i>A. terreus</i> Thom	2	3			3	10	18	24.7
<i>A. versicolor</i> (Vuill.) Tirab	1		3			3	7	9.6
<i>Penicillium</i>						5	5	6.8
<i>P. chrysogenum</i> Thom						3	3	4.1
<i>P. citrinum</i> Thom						2	2	2.7
<i>Chaetomium globosum</i> Kunze ex. Fries		1	2			1	4	5.5
<i>Cladosporium herbarum</i> (Pers) Link						2	2	2.7
<i>Rhizopus</i>			2	2			4	5.5
<i>R. nigricans</i> Ehrenberg			2	1			3	4.1
<i>R. oryzae</i> Went & Prins. Geerl				1			1	1.4
<i>Phoma leveillei</i> Boerema & Bollen			4	2			6	8.1
Total Count	3	4	18	8	5	35	73	100
Number of genera = 6	1	2	4	3	1	4		
Number of species = 13	2	2	7	5	2	10		
Total count	7			31		35	73	100
Number of genera	3			4		4		
Number of species	4			10		10		

Sh = eye shadows

M = eye Mascara

C = cream (face cream cosmetic)

P = powdered (face powdered cosmetic)

F = foundation

Lip = rouges + lip sticks + lip glosses cosmetic products)

*PDA = Potato Dextrose Agar

were encountered and constituted 5.5% of total fungi. *Cladosporium herbarum* was encountered at a low frequency which constituted 2.7% of total fungi (Table-1).

Occurrence of thermophilic fungi at 45±2°C

At 45±2°C, 24 species and two species varieties belonging to 11 genera of thermophilic or thermo tolerant fungi were isolated from the opened cosmetic products samples (Table 2).

The highest total counts 53 of fungi was obtained from the lip cosmetic products, followed by the samples of face cosmetic products (36), while the lowest total counts was obtained from the samples of eye cosmetic products (20).

Aspergillus was the genus most commonly recovered

(78% of total fungi). It was represented by 12 species and one species variety of which *A. terreus* was the most frequent species. This species came ahead of all *Aspergillus* as well as other fungal species in both frequency (36.7% of total fungi). *A. niger* was the second species, contributing (12.8% of total fungi). The other remaining *Aspergillus* species were of low occurrence (Table 2). Among the true thermophilic fungal species although occurred in low incidence (0.9% of total fungi), but *Myriococcum albomyces* and *Rhizomucor pusillus* were isolated (3.7% and 4.6% of total fungi respectively). Both *Chaetomium* and *Rhizopus* were encountered in 5.5% and 2.8% of total count fungi respectively. The rest of fungal genera and species listed in Table (2) were of low frequencies.

Table 2. Total counts and percentage of fungi isolated on PDA* medium at 45°C from cosmetic of eye, face and lip products.

Genera and species of fungi	Eye		Face			Lip	Total count	% of T.C.
	Sh	M	C	P	F			
<i>Aspergillus</i>	12	3	5	18	1	46	85	0.78
<i>A. chevalieri</i> (Mangin) Thom & Church				1			1	0.9
<i>A. clavatus</i> Desmazieres						1	1	0.9
<i>A. deflectus</i> Fennel&Raper						1	1	0.9
<i>A. flavus</i> Link				5		2	7	6.4
<i>A. flavus</i> var. <i>columnaris</i> Raper & Fennell				2			2	1.8
<i>A. fumigatus</i> Fresen.	1	1	3			2	7	6.4
<i>A. niger</i> Tiegh.				5		9	14	12.8
<i>A. sydawi</i> (Bain. & Sart.) Thom & Church				1			1	0.9
<i>A. tamarii</i> Kita						1	1	0.9
<i>A. terreus</i> Thom	10	2	1	4	1	22	40	36.7
<i>A. ustus</i> (Bainier) Thom & Church						2	2	1.8
<i>A. versicolor</i> (Vuill.) Tiraboschi	1		1				2	1.8
<i>A. wentii</i> Whemer						6	6	5.5
<i>Emericella rugulosa</i>				1			1	0.9
<i>Talaromyces thermophilus</i> Stolk			1				1	0.9
<i>Chaetomium</i>	1	2	1			2	6	5.5
<i>Chaet. globosum</i> Kunze ex. Fries						1	1	0.9
<i>Chaet. thermophilus</i> var. <i>dissitum</i> La louch	1	2	1			1	5	4.6
<i>Cladosporium sphaerospermum</i> Penzig				1			1	0.9
<i>Mucor miehei</i> ex Fries	1						1	0.9
<i>Myriococcum albomyces</i> Fries			3			1	4	3.7
<i>Rhizomucor pusillus</i> Lindt (Schipper)	1			2		2	5	4.6
<i>Rhizopus</i>			2			1	3	2.8
<i>R. arrhizus</i> Fisher						1	1	0.9
<i>R. nigricans</i> Ehrenberg			1				1	0.9
<i>R. stolonifer</i> Ehrenb. ex. Fr. Lindt			1				1	0.9
<i>Phoma leveillei</i> Boerema & Bollen				1			1	0.9
<i>Thermoascus thermophilus</i> (Sopp) Von Arx						1	1	0.9
Number of genera = 11	4	2	5	5	1	6	109	
Number of species = 24 + 2	6	3	8	10	1	15		
Total count	15	5	12	23	1	53		
Number of genera	4			9		6		
Number of species	6			17		15		
Total count	20			36		53		

Sh = eye shadows

M = eye Mascara

C = cream (face cream cosmetic)

P = powdered (face powdered cosmetic)

F = foundation

Lip = rouges + lip sticks + lip glosses cosmetic products).

*PDA = Potato Dextrose Agar

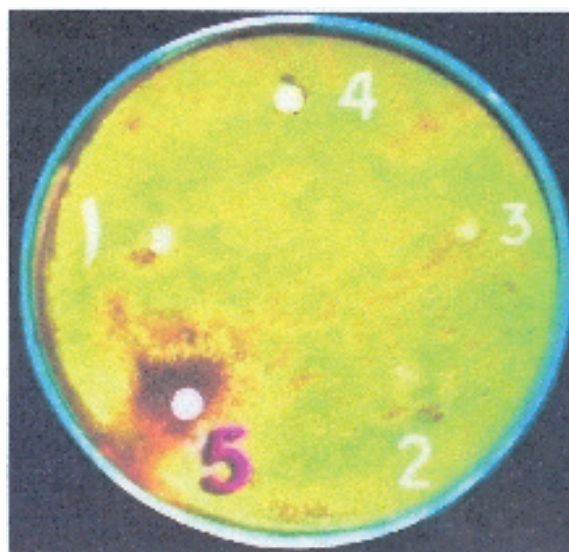
Antifungal activity

The results presented in Table (3) and (Fig. 1) show the antifungal activity of seven randomly selected commercial drugs four of them were skin cream antibiotics (Dermocort, Kena comb, Fucidin and Fusi-zon) and three were drugs used for eye infections (one ointment terramycin, & 2 eye drops, Isopto fenicol 0.5% drops-5 and Neo-pol eye drops were tested against certain recovered fungal isolates.

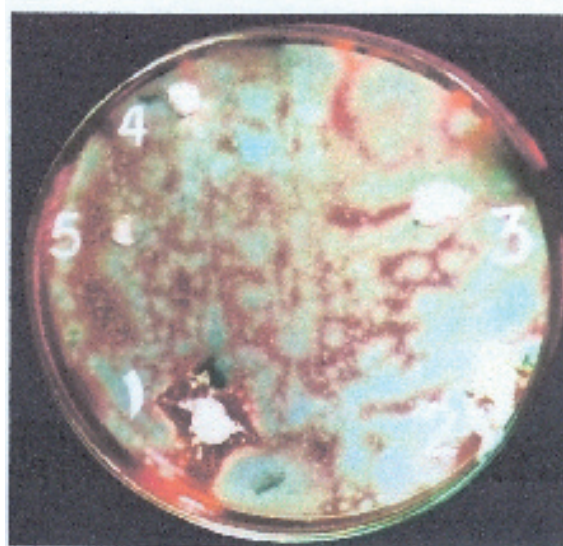
As presented in table (3) and Fig (1) drug (1) Showed inhibitory effect against *Aspergillus flavus*, *A. fumigatus*, *A. terreus*, *A. versicolor*, and *penicillium chrysogenum*, while drug (4) gave inhibitory effect against *A. flavus* and *A. versicolor*, Meanwhile drug 5 and 7 should inhibitor effect against *A. flavus* only. Other drugs showed no inhibitory effect on all tested fungi.

Discussion

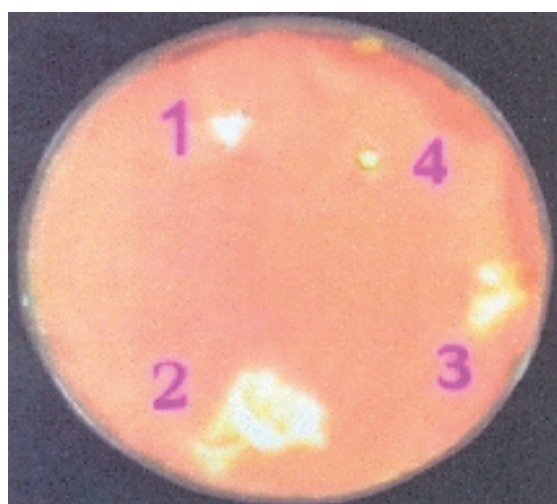
Seventy-three fungal isolates, representing 6 genera and 13 species were surveyed at 28°C in 75 samples of used-cosmetics that were open and used for varying periods of time were collected from different localities in Saudi Arabian. Most of the isolated fungi here had been reported before from cosmetics (Jasnow and Smith, 1975; Mislivec *et al.*, 1993; Ozyaral *et al.*, 1993 and Fujita *et al.*, 2005). Plate counts of zero were found for 60% of samples incubated at 28°C and 20% of samples incubated at 45°C. Mislivec *et al.* (1993) recorded that plate counts of 0 were found for 91.8% of contamination level per product type.



(A)



(B)



(C)

Fig 1. Activity of tested drugs (See table 3) against *Aspergillus flavus* (A) *A. fumigatus* (B) and *A. terreus* (C).

Genera isolated at 28°C were *Aspergillus*, *Phoma*, *Penicillium*, *Chaetomium*, *Rhizopus* and *Cladosporium*. *Aspergillus* was the most common genus it was represented by 6 species at 28°C, whereas Mislivec *et al.* (1993) isolated 13 species of *Aspergillus* and 18 species of *Penicillium*, while in this study *Penicillium* was represented by two species only at 28°C. *Aspergillus terreus* was the most common species (Table 1). *Phoma levulleii* was the second, while *Penicillium* occupied the third place, whereas it was the first of occurrence in Mislivec *et al.* (1993).

Table 3. Activity of drugs tested (1-7) on the growth of different fungal isolates.

Drug fungus	1	2	3	4	5	6	7
<i>Aspergillus</i>							
<i>A. flavus</i>	(+)	(-)	(-)	(+)	(+)	(-)	(+)
<i>A. fumigatus</i>	(+)	(-)	(-)	(-)	(-)	(-)	(-)
<i>A. terreus</i>	(+)	(-)	(-)	(-)	(-)	(-)	(-)
<i>A. versicolor</i>	(+)	(-)	(-)	(+)	(-)	(-)	(-)
<i>Penicillium chrysogenum</i>	(+)	(-)	(-)	(-)	(-)	(-)	(-)

No. 1= Dermocort cream (for skin).

2= Kena comb cream (for skin)

3= Fucidin cream (for skin)

4= Fusi-zon cream (for skin)

5= Isopto fenicol 0.5% eye drops

6= Terramycin ointment for eye

7= Neo pol eye drops

(+) = Inhibition zone

(-) = No zone of inhibition

Concerning the pathogenicity of *Aspergillus* species *A. terreus* was isolated from mycotic abscesses of humans by (Sukroongreung & Thakerngal, 1985). *A. fumigatus* was reported to cause pulmonary aspergilloma by (Arianayagam *et al.*, 1986) and Onychomycoses by (Van Bersusegher, 1952).

A. species and two species varieties belonging to eleven genera of thermophilic and thermotolerant fungi were collected. *Aspergillus* was the most common genus contributed the greatest number of species (12 species & one species variety). *A. terreus* also was the most common species as in mesophilic fungi study.

A. niger was the second species of *Aspergillus* at 28 and 45°C while *A. flavus* and *A. fumigatus* were the third. *A. fumigatus* was considered by Cooney & Emerson (1964) to be thermotolerant as it has a maximum temperature for growth near 50°C, but the minimum was below 20°C. *A.* cosmetics and/or toiletry products maybe exposed to higher temperature than recommended which can also accelerate the growth rate of microbes (Daniel, 2007).

From the antifungal activity that was studied the results indicated that Dermocort skin cream was largely effective against all tested fungal isolates in this study. Sutton *et al.* (1998) reported that many other fungal isolates have been tested for antifungal susceptibility in vitro.

The proportion of fungal isolates from lip cosmetic products was higher and the proportion from eye products was lower in this study than would be expected from the samples distribution. Whereas in (Mislivec *et al.*, 1993) reported that the proportion of eye products was higher than that of lip products.

The contamination of cosmetics by fungi is due to the quality of raw materials used in its manufacture as in (Jasnow & Smith, 1975). Also, fungi can cause spoilage problems in certain finished products as reported by (Bonorden, 1973). On the other hand, from this study cosmetics contamination may be due to fungal air spore and human hand contamination after using for several time of survival opening.

Also misuse and old cosmetics can harbor dangerous bacteria and fungi and can cause eye infection as reported by (Carol Belanger, 2007 and Klotz *et al.*, 2000). Patricia Davis (2007) reported several guidelines for safe cosmetic use.

- i. Wash your hands and face before applying cosmetics.
- ii. which included Eye cosmetics should be thrown away after 6 months.
- iii. And Mascara should be thrown away after 3 months.

Finally it is recommended to throw away old cosmetics and never share cosmetics with another person and these precautions could save you unnecessary aggravation and a potentially serious infection.

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نمط الفطريات المعزولة من مستحضرات التجميل المفتوحة والمستعملة من أماكن مختلفة بالمملكة العربية السعودية

ليلى أحمد ناصر

جامعة الرياض للبنات - المملكة العربية السعودية

المخلص

تم تجميع 75 عينة من مستحضرات التجميل المفتوحة والمستعملة على فترات مختلفة من أماكن مختلفة بالمملكة العربية السعودية تم تحليلهم لوجود الفطريات المتوسطة والمحبة للحرارة عند درجات الحرارة 28° و 45° م. وتم عزل تسعة أجناس وثلاثة عشر نوعاً من الفطريات عند درجة حرارة 28° م. كما تم عزل إحدى عشر جنساً وأربعة وعشرون نوعاً وسلالتين عند درجة الحرارة 45° م (للفطريات المحبة للحرارة). كما تم الحصول على أعلى عدد من الفطريات من العينات المعزولة من مستحضرات التجميل للشفاة وأقل عدد للفطريات من العينات المعزولة من مستحضرات التجميل للعين. كما تم اختيار قدرة سبعة عقاقير تجارية مختارة عشوائياً على تثبيط أو عدم تثبيط الفطريات المعزولة وأوضحت النتائج أن عقار ديرموكورت كريم (للجلد) ، ذات كفاءة عالية ضد العزلات الفطرية المستخدمة.