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# Comparative Microbiological Study between Traditional and Modern Cosmetics in Saudi Arabia

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## Abstract

Cosmetic products support microbial growth due to the presence of variable amounts of nutrients. The most bacterial contaminants that were found in cosmetic products *Staphylococcus, Pseudomonas, Klebsiella, Achromobacter* and *Alcaligenes*. Mostly due to contaminated water. So this study aimed to determine and compare between the microbial contamination of traditional products such as Athmad *(kohl),* Henna (*Lawsonia inermis*), *(Ocimum),* Sedr *(Rhamnu*s), Musk, Derum *(Juglan regia* L.), Mshat *(Alcea)* and Magic rouge in addition to modern cosmetic products from cheap and valuable trade mark such as Mascara, Eyeliner, Rouge, Plusher, Face powder and Foundation in two different states of use (intact and in-use). In this study, 67 traditional and modern cosmetic products analyzed microbiologically, the result revealed that *Salmonella* was the predominant isolates from intact and used collected samples with an equal incidence 76% equal to the incidence of *Staph. epidermis* from used samples followed by *Staph. epidermis* with an incidence of 57% from intact isolates while the incidences of *Staph aureus* were 43% and 16% from intact and used samples respectively. Among intact and used samples *E. coli* was isolated from only 2 samples with low incidence 0.02% and 0.04% respectively. The incidence of microbial contamination was higher in modern cosmetics than traditional cosmetics especially in Athmed *(kohl)* samples, also microbial contamination was high in incidence in mascara, plusher and eye shadow as modern cosmetics , so it could be concluded that cosmetic products produced in Riyadh , can be contaminated during the production process and they can serve as vehicles for the transmission of these pathogenic organisms . Therefore it is important to take precautions during production process in order to prevent infections due to microbial contamination.

## Introduction

The Ministry of Health of Turkey defined "Cosmetics as all the preparations that were prepared to be used for epidermis, nails, hair, lips, genital organs and teeth and mouth mucosa and their only aim is to clean, give odors, change the morphological appearance and/ or to regulate the body odors and keep them in good positions" [1]. However the Federal Food and Drug Cosmetic Act criteria defined it as the articles intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body or any part thereof for cleansing, beautifying, promoting attractiveness, or altering the appearance, and articles intended for use as a component of any such articles; except that such term shall not include soap [2]. Cosmetics microbial contamination may lead to spoilage of the product and when pathogenic and act as serious health risk for consumers [3] cosmetics mostly are not sterile and manufactured of non-sterile raw material [4-6]. However cosmetics do not have to be sterile, limit values have been reported according to the type of the cosmetics [5]. So these preparations should follow the Good Manufacturing Practice (GMP) rules [1] Limits of microorganisms that can be found in cosmetic preparations such as *Staphylococcus aureus, Escherichia coli, Salmonella spp.,Candida albicans, Clostridium spp.,* and *Pseudomonas aeruginosa.* Should be limited and mentioned. For example; 500 CFU/g in cosmetics that are used for eye area, 1000 CFU/g in other cosmetics in 1g or 1ml of the preparation [6]. Due to this reason it is important to investigate the microbial content of the cosmetic preparations, firstly according to the aerobic microorganism number in 1g or 1ml of the sample, secondly according to the existence of some specific microorganisms such as *S. aureus, P. aeruginosa,* and *C. albicans.* Also to control the microbial growth and to stabilize any cosmetic product, some preservative needs to be used. However, in many cosmetics there is no reported expiry date which act as health potential risk due to lose of

the preservative activity [7]. Before the 1930's the field of cosmetics and microbiology had not come into contact, however in 1940s cosmetic microbiology became more important [8]. The first reported cosmetics contamination was in 1946 due to several cases of neonatal death from talcum powder containing *Clostridium tetani* [9]. Since 1960's, a lot of opportunist organisms have been isolated from cosmetic products, such as *Klebsiella pneumoniae, Pseudomonas aeruginosa, Pseudomonas sp., Serratia sp.* and *Enterobacter sp.* [9,10]. However the sterilization of cosmetic products are not to be accepted but it is important be free of pathogenic microorganisms (like *Staphylococcus aureus, Escherichia coli, P. aeruginosa*) and with low total aerobic microbial count**.** [11,12]. The presence of pathogenic microorganisms with high levels in cosmetic products lead to spoilage (physical change of the product) which act as health hazard for consumers [3,13]**.** And also it is important to improve the preservative system in order to inhibit the growth of contaminating microorganisms during production, storage and use by consumers [14]. So this study aimed to determine and compare the microbial contamination of traditional products such as Athmad (kohl), Henna (*Lawsonia inermis*), *(Ocimum),* Sedr *(Rhamnus)*, Musk, Derum (*Juglan regia* L.), Mshat *(Alcea)* and Magic rouge in addition to modern cosmetic products from cheap and valuable brands such as Mascara, Eyeliner, Rouge, Plusher, Face powder and Foundation in two different states of use (intact and in-use).

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| Types of samples | | Intact | Used | Total | |
|---|---|---|---|---|---|
| **Traditional** | Mshat *(Alcea)* | 1 | 0 | 1 | 32 |
| | Henna *(Lawsonia inermis)* | 1 | 0 | 1 | |
| | Nail henna | 1 | 0 | 1 | |
| | Rehaan *(Ocimum)* | 1 | 0 | 1 | |
| | *Derum Juglan regia* L | 1 | 1 | 2 | |
| | Musk | 1 | 1 | 2 | |
| | Sedr *(Rhamnus)* | 1 | 0 | 1 | |
| | Athmad *(kohl)* | 10 | 0 | 10 | |
| | Henna perfume | 2 | 0 | 2 | |
| | Magic rouge | 1 | 1 | 2 | |
| **Modern** | Plusher | 4 | 6 | 10 | 44 |
| | Powder | 2 | 0 | 2 | |
| | Foundation | 2 | 0 | 2 | |
| | Eye liner | 4 | 3 | 7 | |
| | Mascara | 5 | 6 | 11 | |
| | Rouge | 3 | 3 | 6 | |
| | Eye shadow | 2 | 3 | 5 | |
| | Eye brow | 0 | 1 | 1 | |
| **Total** | | 42 | 25 | 67 | |

**Table 1**: Number and types of samples collected from cosmetics.

| Micro-organisms | Traditional | | Modern | | Intact | | Used | |
|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | No. | % |
| *Salmonella* | 61 | 77% | 43 | 77% | 32 | 76% | 19 | 76% |
| *E. coli* | 4 | 64% | 7 | 7% | 1 | 0.02% | 1 | 0.04% |
| *Staph. epidermis* | 66 | 34% | 43 | 74% | 24 | 57% | 19 | 76% |
| *Staph. aureus* | 66 | 16% | 3 | 6% | 18 | 43% | 4 | 16% |

**Table 2:** The prevalence of microorganisms among the traditional and modern, used and intact cosmetics.

## Materials and Methods

### Cosmetic products

Sixty-seven commercially available cosmetic products in two different states of use, the intact product (at the time of purchase) were purchased from Riyadh markets, the in-use product (after 14 days of use) , were collected and analyzed in order to determine the microbiological contamination during the manufacturing or during their use by consumers. *Escherichia coli, P. aeruginosa, Salmonella spp., S. aureus and Enterobacteria*, were the investigated microbial species as suggested by USP and EP. Consumers, randomly selected among the students of Shaqraa University, The sample contain (1 ml of liquid product or 1 g of paste) of the cosmetic product, collected it in a sterile tube, in the two states mentioned above. Microbiological analyses such as isolation and identification of bacteria were performed on the collected cosmetic samples represented in Table 1.

## Microbiological Analyses

### Media and isolation of pathogenic micro-organisms

To determine the presence of pathogenic micro-organisms, Sterile swabs from extracted samples(powder samples)and directly from ( rouge and musk) were spread on Mannitol salt agar (Watin ), McConkey (Watin) and nutrient agar (Watin) to allow the growth of *Staphylococcuss* pp., *Enterobacteria* and *Pseudomonas spp*. respectively. The plates were then incubated at 37°C for 24 h. Isolates were identified by conventional biochemical tests [15,16].

## Results

Table 2 revealed that 16,3,11 and 15 out of 44 collected traditional cosmetic samples were positive to *Salmonella, E. coli, Staph. epidermis and Staph. aureus* respectively with an incidence of 70%, 13%, 48% and 65% respectively while 34,0,32 and 4 out of 23 collected modern cosmetic samples were positive to *Salmonella, E. coli, Staph. epidermis*

| Different Data about Cosmetics | | Types of samples | No. of different samples | Different isolated M.O. | | | |
|---|---|---|---|---|---|---|---|
| | | | | Salmonella | E.coli | Staph epidermis | Staph aureus |
| **Edition** | **Traditional** | Mshat (Alcea) | 1 | 1 | 0 | 0 | 0 |
| | | Henna(Lawsonia inermis) | 1 | 1 | 0 | 0 | 1 |
| | | Nail henna | 1 | 0 | 0 | 1 | 0 |
| | | Rehaan (Ocimum) | 1 | 1 | 1 | 0 | 1 |
| | | Derum Juglan regia L | 2 | 1 | 0 | 0 | 1 |
| | | Musk | 2 | 0 | 2 | 0 | 0 |
| | | Sedr (Rhamnus) | 1 | 1 | 0 | 0 | 1 |
| | | Athmad(kohl) | 10 | 10 | 0 | 10 | 10 |
| | | Henna perfume | 2 | 0 | 0 | 0 | 0 |
| | | Magic rouge | 2 | 0 | 0 | 1 | 0 |
| | **Modern** | Plusher | 10 | 10 | 0 | 6 | 0 |
| | | Bowder | 2 | 2 | 0 | 2 | 2 |
| | | Foundation | 2 | 2 | 0 | 0 | 0 |
| | | Eye liner | 7 | 7 | 0 | 7 | 0 |
| | | Mascara | 11 | 11 | 0 | 11 | 0 |
| | | Rouge | 6 | 0 | 0 | 0 | 0 |
| | | Eye shadow | 5 | 3 | 0 | 5 | 5 |
| | | Eye brow | 1 | 0 | 0 | 0 | 0 |

| Usage | | | | | | | |
|---|---|---|---|---|---|---|---|
| Usage | Intact | Mshat (Alcea) | 1 | 1 | 0 | 0 | 0 |
| | | Henna(Lawsonia inermis) | 1 | 1 | 0 | 0 | 1 |
| | | Nail henna | 1 | 0 | 0 | 1 | 0 |
| | | Rehaan (Ocimum) | 1 | 1 | 1 | 0 | 1 |
| | | Derum Juglan regia L | 1 | 1 | 0 | 0 | 1 |
| | | Musk | 1 | 0 | 1 | 0 | 0 |
| | | Sedr (Rhamnus) | 1 | 1 | 0 | 0 | 1 |
| | | Athmad(kohl) | 10 | 10 | 0 | 10 | 10 |
| | | Henna perfume | 2 | 0 | 0 | 0 | 0 |
| | | Magic rouge | 1 | 0 | 0 | 0 | 0 |
| | | Plusher | 4 | 4 | 0 | 0 | 0 |
| | | Bowder | 2 | 2 | 0 | 2 | 2 |
| | | Foundation | 2 | 2 | 0 | 0 | 0 |
| | | Eye liner | 4 | 4 | 0 | 4 | 0 |
| | | Mascara | 5 | 5 | 0 | 5 | 0 |
| | | Rouge | 3 | 0 | 0 | 0 | 0 |
| | | Eye shadow | 2 | 0 | 0 | 2 | 2 |
| | | Eye brow | 0 | 0 | 0 | 0 | 0 |
| | Used | Mshat (Alcea) | 0 | 0 | 0 | 0 | 0 |
| | | Henna(Lawsonia inermis) | 0 | 0 | 0 | 0 | 0 |
| | | Nail henna | 0 | 0 | 0 | 0 | 0 |
| | | Rehaan (Ocimum) | 0 | 0 | 0 | 0 | 0 |
| | | Derum Juglan regia L | 1 | 1 | 0 | 0 | 1 |
| | | Musk | 1 | 0 | 1 | 0 | 0 |
| | | Sedr (Rhamnus) | 0 | 0 | 0 | 0 | 0 |
| | | Athmad(kohl) | 0 | 0 | 0 | 0 | 0 |
| | | Henna perfume | 0 | 0 | 0 | 0 | 0 |
| | | Magic rouge | 1 | 0 | 0 | 1 | 0 |
| | | Plusher | 6 | 6 | 0 | 6 | 0 |
| | | Bowder | 0 | 0 | 0 | 0 | 0 |
| | | Foundation | 0 | 0 | 0 | 0 | 0 |
| | | Eye liner | 3 | 3 | 0 | 3 | 0 |
| | | Mascara | 6 | 6 | 0 | 6 | 0 |
| | | Rouge | 3 | 0 | 0 | 0 | 0 |
| | | Eye shadow | 3 | 3 | 0 | 3 | 3 |
| | | Eye brow | 1 | 0 | 0 | 0 | 0 |

**Table 3:** Collective table and the incidence of different isolated microbes.

and *Staph. aureus* respectively with an incidence of 77%, 0% , 73% and 1% in addition to that *Salmonella* was the predominant isolates from intact and used collected samples with an equal incidence 76% equal to the incidence of *Staph. epidermis* from used samples followed by *Staph. epidermis* with an incidence of 57% from intact isolates while the incidences of *Staph. aureus* were 43% and 16% from intact and used samples respectively. Among intact and used samples *E. coli* was isolated fom only 2 samples with low incidence 0.02% and 0.04% respectively.

The incidence of different isolated microorganisms regarding to different collected cosmetic samples were illustrated in Table 3. *Salmonella* was the predominant isolate from all collected samples followed by *Staph. epidermis* followed by *Staph. aureus* and the lowest incidence regarding to *E. coli* isolates and the incidence of microbial contamination was higher in modern cosmetics than traditional cosmetics especially in Athmed *(kohl)* samples, also microbial contamination was high in incidence in mascara, plusher and eye shadow as modern cosmetics (Table 3).

## Discussion

The microbial limit standards of a non-sterile product, such as cosmetic formulations, was demonstrated in (Official Italian Pharmacopeia: [17,18]. These values should be written in the products during their use, in spite of the addition of a suitable preservative in the products to limit the contamination by the users in addition to control the microbial during the storage before marketing [19]. However, the literature regarding the efficacy of preservative systems contained in cosmetic products to control the microbial contamination of these products during their use by consumers with poor information [14,20]. Pathogenic microbial contamination of cosmetics leads to spoilage of the product and act as serious health risk for consumers [3].

According to a 1989 FDA report on contamination of makeup counter samples in department stores "Cosmetics are not expected to be totally free of microorganisms when first used or to remain free during consumer use," [4]. There are several previous studies have done to investigate the microbial contamination of some unused cosmetics as

which was done by Altanla on 81 unused lipstick samples; 34 samples have been found to be contaminated and total aerobic bacteria counts were between 104-106 CFU/g. In addition to some mold and yeast which are not allowed to be present in cosmetics were determined [21]. Also the creams which were prepared by Ege University Department of Chemical Engineering have investigated by Özdemir. Only one of the samples *Staphylococcus aureus* was isolated and no other pathogen bacteria mold or yeasts were observed [22]. 14 unused samples of shampoo, hand cream, hair tonic and hair cream samples were studied by Ergun where *P. aeruginosa*, 2 *E. coli*, 2 *S. aureus*, 5 *Bacillus subtilis*, 2 *Enterobacter spp.* were isolated from the samples [3,23]. 45 unused and 56 used cosmetics samples (shampoos, creams, mascaras and lipsticks) have studied by Anar. Levels of bacterial and fungal contamination were 53.47% and 35.64% respectively. 22 unused and 18 used cosmetic samples had pathogenic microorganisms. 12% of used cosmetics products contained more than 103 CFU/ml org [24] 91 commercially available cosmetic products has been studied by Campana et al. [3] (at the time of purchase), the in-use products (after 14 days of use) and the ending product (post use). In all cases the contamination was found in ending products, however in one case in the in-use product [3]. Ravita et al. [6] have studied with post-consumer use cosmetic products in case of microbial contamination [6]

So this study aimed to determine and compare the microbial contamination of traditional products such as Athmad *(kohl)*, Henna *(Lawsonia inermis)*, (*Ocimum*), Sedr *(Rhamnus)*, Musk, Derum *(Juglan regia* L.), Mshat *(Alcea)* and Magic rouge in addition to modern cosmetic products from cheap and valuable brands such as Mascara, Eyeliner, Rouge, Plusher, Face powder and Foundation in two different states of use (intact and in-use).

The results of the present study revealed that 16,3,11 and 15 out of 44 collected traditional cosmetic samples were positive to *Salmonella, E. coli, Staph. epidermis* and *Staph. aureus* respectively with an incidence of 70%, 13%, 48% and 65% respectively while 34,0,32 and 4 out of 23 collected modern cosmetic samples were positive to to *Salmonella, E. coli, Staph epidermis* and *Staph aureus* respectively with an incidence of 77%, 0%, 73% and 1% in addition to that *Salmonella* was the predominant isolates from intact and used collected samples with an equal incidence 76% equal to the incidence of *Staph. epidermis* from used samples followed by *Staph. epidermis* with an incidence of 57% from intact isolates while the incidences of *Staph. aureus* were 43% and 16% from intact and used samples respectively. Among intact and used samples *E. coli* was isolated from only 2 samples with low incidence 0.02% and 0.04% respectively as shown in Table 2.

Results from this study showed that bacteria isolated from various cosmetic sources were identified on the basis of growth on nutrient agar (primary identification), microscopy, growth on selective media (secondary identification) and finally biochemical analysis. The four different bacteria were isolated from all cosmetics samples and no microorganisms were isolated from Lipstick and perfumes. The most frequently identified microorganism was *Salmonella* which is the most common due to water contamination followed by Staph epidermis followed by *Staphylococcus aureus*, which is the most common bacterial skin pathogen. Cosmetics application is largely restricted to the skin. *Staphylococcus aureus* is a common skin microorganism that can cause boils, impetigo, conjunctivitis, folliculitis and food poisoning [25].

Previous studies on cosmetic products also had similar results. A survey on cosmetics microbial contamination was done in Europe between 2005-2008. 24 different cosmetic products were reported to be contaminated with *Pseudomonas aaeruginosa, Bacillus cepacia,* *Staphylococcus aureus, Enterococcus sp.* etc. [24]. Similar results showed 10 commercially available cosmetic creams and lotions which were purchased and their microbiological contents were evaluated. Investigators identified *Staphylococcus aureus, Streptococcus sp. and Bacillus sp.* similar to previous studies [26-28].

According to microbiological standards of cosmetic products, they must be free of high virulence microbial pathogens like *S. aureus* and *P. aeruginosa;* however, the present study results revealed that these microorganisms can be found in unused cosmetic products. Most of the samples were contaminated by *E. coli* and *Salmonella sp.* These microorganisms are also known to be opportunistic, with some of them resistant to microbial agents and they can also cause infections to immunosuppressive patients [29-31]. In addition to outbreak investigations which resulted in the demonstration of these opportunistic pathogens in contaminated cosmetic products [32]. Sanitary processing and using appropriate and adequate preservatives can control the microbial contamination, from manufacturer to consumer, especially *Staphylococcus aureus, Salmonella* and *E. coli* that were not allowed to be found in cosmetics .

The pH of all the tested samples was alkaline pH (8.2-9), which inhibit fungal contamination and growth. Bacterial contamination in unused cosmetic products is common because of the environment in which the products are manufactured, packed due to the organic substrates which present in it such as sugar, starch, protein, amino acid, organic acid, alcohol, amines, lipid and etc. Which help the microbial growth? Malcom and Woodroffe [33] reported that the most frequent contaminants of cosmetic products are *Pseudomonas, Klebsiella, Achromobacter* and *Alcaligenes* which are common residents in contaminated water as it is a likely source of the organisms found in contaminated cosmetic products. [34,35]The obtained results are similar or like the report of [20] Gram negative bacilli were seen in these studies, but unlike the report of Hugbo et al. [27]. Unlike the report of Altanlar N [20,27]. *Salmonella* spp. was isolated in the present study. And generally, microorganisms of interest in raw materials as in cosmetic products especially in neutral pH 7.0 and many yeasts and moulds are able to tolerate acid pH conditions. Therefore, cosmetics should be produced in a perfectly clean hygienic environment and follow guidelines of good manufacturing practice (GMP). All starting materials should be of good quality [36]. Ingredient listing is an important aspect of the labelling of cosmetic products. During the Nance's Pharmaceutical Control Bureau Cosmetics Seminar 2002, one of the requirements discussed was labelling of cosmetic products [37]

In accordance the incidence of microbial contamination was higher in modern cosmetics than traditional cosmetics especially in Athmed *(kohl)* samples, also microbial contamination was high in incidence in mascara, plusher and eye shadow as modern cosmetics as it was shown in Table 3.

From the previous results it was clear that bacterial contamination is more likely to occur than yeast and mould contamination. Bacterial growth is favoured at neutral pH and most cosmetic products are at this range. Microorganisms such as *salmonella, staph epidermis, staph aureus and E. coli* are the most frequently reported contaminants of cosmetic products. Also, contamination is higher in Athmed *(kohl)* than other products. This may be because they contain surfactants.

Which are susceptible to contamination by water-borne Gram-negative bacteria? Several cases of eye infections and even loss of vision were also caused by contaminated cosmetic products contaminated with *P. aeruginosa* [38,39].

## Conclusion

It can be concluded from the findings of this research work that cosmetic products produeid in Riyadh, can be contaminated during the production process. The presence of organisms such as *Salmonella, Staphylococcus aureus* and *E. coli* in the cosmetic collected samples that they can serve as vehicles for the transmission of these pathogenic organisms.

Therefore it is important to take precautions during production process in order to prevent infections due to microbial contamination. It is necessary to comply with GMP standards strictly during the production. Preservatives should be added to products as determined by regulation and in accordance with toxic dose limits, for consumer's health. A further study on preservatives will be carried out to detect the preservative level in cosmetic products marketed in Riyadh.

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