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A Study of Microbiological Contamination in Cosmetics and Toiletries in Iraq. Contamination of Talcum Powder and Body Lotion

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الخلاصة

تم دراسة حوالي 100 مائة نوع من المستحضرات الصيدلانية التي تشمل (مسحوق التالكوم وغسول الجسم)، حيث تم اخذ (ستون نموج من مسحوق التالكوم، اربعون من غسول الجسم) ودراسة مافيها من البكتريا الهوائية، البكتريا المعوية وكذلك وجود الفطريات والبكتريا اللاهوائية بالنسبة لمسحوق الـ (Talcum) . وقد تم ايضاً تشخيص انواع من البكتريا المرضية.

وجد من خلال هذه الدراسة ان (مسحوق الـ Talcum) اكثر تلوثا بالبكتريا من غسول الجسم (حوالي 60% من غسول الجسم لا تحتوي على بكتريا او احتوائها على عدد اقل من 100 C.F.U/gm بينما 35% من مسحوق التالكوم يحوي هذه النسبة) وان (5% من مسحوق الـ Talcum) يحوي على نسبة 10^4 C.F.U للغرام الواحد بينما غسول الجسم لا تحوي هذه النسبة العالية من البكتريا. كما انه لا تحوي غسول الجسم على البكتريا المعوية بينما 3.3% من مسحوق الـ Talcum يحوي هذه البكتريا بمعدل 250−300 C.F.U للغرام الواحد.

تم تشخيص بكتريا (Staphylococcus) في نموذج واحد من كل من مسحوق الـ Talcum وغسول الجسم. وان جميع النماذج او العينات التي اخذت سواء من مسحوق الـ Talcum أو غسول الجسم لاتحوي اي نوع من انواع الفطريات وان مسحوق الـ Talcum اظهر عدم وجود اي من البكتريا اللاهوائية (anaerobic bacteria).

Abstract:

One hundred items of 60 talcum powders and 40 body lotions were examined for their total aerobic bacterial, coliform and fungal counts. We also carried out anaerobic bacterial counts for talcum powder as well as tests to detect some potentially hazardous bacteria in all tested samples. Talcum powders were more heavily contaminated with bacteria than body lotions. More than 60% of the tested body lotions contained no viable bacteria or less than 100 C.F.U./gm, while 35% of the talcum powders contained this level. Five per percent of the talcum powder were contaminated with 10^4 C.F.U. /gm and none of the body lotions were contaminated to that extent. No coliforms were recovered from any of the body lotions, while 3.3% of the talcum powder

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examined contained coliforms in the range of 250-300 C.F.U/gm. Staphylococcus Spp. were detected in one sample of both talcum powders and body lotions. Two samples of talcum powder contained E. coli. Neither talcum powder nor body lotions showed fungal counts. Also no talcum powder showed the presence of anaerobic bacterial counts.

Keyword: microbial contamination, cosmetics, talcum powder, body lotion.

Introduction:

Microbial spoilage of different items such as food, papers, and textiles, has been known for many years. It is perhaps a little surprising that the problem of microbial contamination in nonsterile medicines and cosmetics received detailed attention only recently[1]. This possibly is due to over confidence in the traditionally good hygienic conditions under which such products are manufactured and also because it is assumed that added preservative will prevent microbial growth upon storage and/or during use[2]. However, studies have shown that although many cosmetic preparations contain preservatives, microbial spoilage can still occur during storage or use [3-5]. The warm and rather humid climatic conditions that prevail in most tropical countries would tend to support the survival and growth of many microorganisms. In a situation where by a nutritionally rich pharmaceutical/cosmetic product is severely contaminated, rapid growth and multiplication would be expected. This could lead to biodegradation of the product and hence the risk of infection to consumers of the product[6].

Product contamination may arise from raw materials or water used in formulation or accidentally, during use, studies carried out till date to assess the incidence and hygienic status of many topical products [7-11, 14]. Using cosmetic preparations which are contaminated with microorganisms has been associated with several diseases. For example, four babies died from Clostridium tetani infections attributed to the use of a talcum powder contaminated with this organism[12]. Wilson and Ahearn [13] have demonstrated that eye cosmetics may serve as a possible medium in transmission and persistence of microorganisms in clinical eye infections. As a part of a comprehensive survey for the microbial contamination of cosmetic preparations manufactured and/or used in Iraq, this study describes the results of a qualitation and quantitative investigations of the microbial content of six commonly used talcum powder and four popular body lotions.

Materials and Methods:

Tested preparations:

Six different talcum powder and four body lotions were examined. A total of ten samples from each preparation were purchased at retail outlets in Iraq.

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Media:

The following dehydrated media (oxoid,ltd, England) were used during the course of the study: tryptic soy agar (TSA), sabouraud's dextrose agar (SDA), MacConkey agar and broth, cosin methylene blue agar (EMB), mannitol salt agar, cooked meat media, reinforced clostridial medium.

* Quantitative determination of viable microorganism viable counts for total bacterial, coliforms, and fungal counts were determined by the conventional pour plate method as described by B.P (2004).

Aerobic plate count:

Sterile materials, equipment and aseptic techniques were used. The caps of the products were wiped with ethanol.

Microbiological media were reconstituted and prepared from their dehydrated powder according to manufacture instructions.

By means of a syringe, spatula, one gram or 1ml of the product was disintegrated in tryptic soy broth (9ml) according to B.P 2004 using a flask shaker and suitable serial dilutions in tryptic soy broth were prepared. One-ml sample of each dilution was poured in a sterile Petridis and them 15ml of sterile tryptic soy agar was poured on the samples, the plates were gently swirled in around movement to allow a good mixing of the agar with sample, then the plates were allowed to solidify on a leveled surface. Triplicate plates for each sample were used and incubated at $35C^0$ for two days for bacteria. Sabourand dextrose agar was used instead of tryptic soy agar-for the detection of fungi. The prepared plates were incubated at $25C^0$ for 5 days. After incubation the numbers of colonies were counted by estimating the total viable aerobic count and total viable count for moulds then the mean of three plates was calculated.

A laboratory control count was performed using negative control blank (without product) and with positive control (contaminated product). More than two colonies on the negative control plate invalidated the test.

The colonies were counted. Colony counts exceeding 1000 were considered too high to count and the product further diluted. Plates with colonies of 30-300 were selected. The microorganism content per milliliter or gram is the colony count multiplied by the appropriate dilution factor (10 or 100).

Detection for specific microorganisms such as Escherichia coli, Staphylococcus aureus, Pseudomonas, and Palmonella was performed following procedure under isolation and identification tests for specified microorganisms (B.P 2004) as shown in table-1. When results showed presence of any of these organisms, appropriate biochemical tests were performed.

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Organism	Enrichment	Primary test	Secondary test	Confirmation
Enterobacteriaceae	Lactose broth 35-37^{0}C	EEB-Mossel 35-37^{0}C For 24-48hr.	VRBGLA 35-37^{0}C	GROWTH OF Gramnegatives
E. coli	As above	MacConkey broth 43-45^{0}C for 18-24hr.	MacConkey agar 43-45^{0}C for 18-24hr.	Indole at 43.5-44.5^{0}C biochemical
Salmonella	As above for 5-24hr.	TBBG broth 42-43^{0}C 18-24hr. then subculture on: DCA.XLDA or BGA for 35-37^{0}C for 24-48hr.	TSI agar 35-37^{0}C for 18-24 hr.	Biochemical serological
Ps.aeruginasa	Saline peptone 35-37^{0}C for 2-5hr	Casein digest broth 35-37^{0}C for 24-48hr.	Cetrimide agar 35-37^{0}C for 24-48hr.	Oxidase test
Staph. aureus	As for Ps.aeroginosa above	As for ps.aeroginosa above	Baird-Parker 35-37^{0}C for 24-48hr.	Coagulase, catalase, DNase tests

Table-1: Isolation and Identification tests for specified microorganisms (BP. 2004)

EEB-m-Mossel. Enterobacteriaceae enrichment broth-Mossel; VRVGLA, violet red agar with glucose and lactose; TBBG, tetrathionate bile brilliant green broth; DCA, deoxycholate citrate agar; XLDA, xylose lysine deoxycholate agar; BGA, brilliant green agar; TSI, triple sugar iron agar; DNase, deoxyribonuclease test.

* Counts of anaerobic spore forming bacteria were made on reinforced Clostridial Agar. The plates were incubated for 3days at 37^{0}C in an atmosphere of 95% hydrogen plus 5% Co_2.

Results:

A total of 100 samples representing six talcum powder and four body lotions were examined for their total aerobic bacterial, coliform, fugal counts. Tests for detection of same potentially hazardous bacteria in the same sample were also carried out. The distribution of the total aerobic bacterial and coliforms counts are summarized in table-2. Asignificant difference (p=o.o5) was noted between the total bacterial count of talcum powder and body lotions. More than 60% the tested body lotions contained fewer than 100 CFU/ml, compared to only 35% of the talcum powders which contained the same count of bacteria/gm. 5% of the tested body lotions contained 10^3-10^4 CFU/ml, compared to 23.33% of the talcum powder similarly, while 5% of the talcum powder were heavily contaminated (more than 10.000 CFU/gm), zero% of the body lotions were heavily contaminated to the same level.

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No coliform bacteria were recovered from any of body lotions, while 3.33% of the examined talcum powder contained coliforms. The range of coliform counts was between 350-300 C.F.U/ml.

Among the talcum powder, brand No. 1 showed the highest contamination, with 70% of the tested samples containing more than 1000 CFU/g. While brand 4 showed the least viable count, a pproximately 70% of the tested samples contaned less than 100 CFU/ml or gm. There were no significant differences in the bacterial counts among the different brands of body lotion tested. Table-2. Qualitative test for the presence of Pseudomonas spp. and Staphylococcus spp. By conventional methods, as well as detection of Escherichia, Salmonellae, proteases were performed on all tested samples. For talcum powder, results showed that one sample contained Staphylococcus spp, which is S. aureus. No sample contained Pseudomonas Spp. and two samples contained E. coli while for body lotions, one sample contained Staphylococcus spp. As shown in table-3.

No yeast or molds contained in both talcum powder and body lotions. With regard to the anaerobic bacterial counts for talcum powder, no samples showed any counts, and C. tetani was not present.

Preparation	No. of items tested	Bacterial count/m 1 or gm	No. and % of items with colony count within the range				No. % of samples>100 coliforms/ml
A. Talcum powder			$0<10^2$	10^2-10^3	$>10^3$-10^4	$>10^4$	
1.	10	5×10^4	0(0)	3(30)	5(50)	2(20)	1(10)
2.	10	9×10^2	4(40)	6(60)	0(0)	0(0)	0(0)
3.	10	1.6×10^3	6(60)	2(20)	2(20)	0(0)	0(0)
4.	10	3.5×10^3	7(70)	2(20)	1(10)	0(0)	1(10)
5.	10	3.2×10^3	0(0)	6(60)	3(30)	1(10)	1(10)
6.	10	3×10^3	4(40)	3(30)	3(30)	0(0)	0(0)
Total	60		21(35)	22(36.6)	14(23.3)	3(5)	3(5)
B. Body lotions							
1.	10	$3x\ 10^3$	4(40)	4(40)	2(20)	0(0)	0(0)
2.	10	$2\ 10^2$	9(90)	1(10)	0(0)	0(0)	0(0)
3.	10	$2x\ 10^2$	8(80)	2(20)	0(0)	0(0)	0(0)
4.	10	$2x10^2$	6(60)	4(40)	0(0)	0(0)	0(0)
Total	40		27(67.5)	11(27. 5)	2(5)	0(0)	0(0)

Table-2: Distribution of Aerobic Bacterial counts in tested preparations.

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Preparation	No. of items tested	No. and % of items containing S.aureus	P. aeuoginosa	Ecoli :
A. Talcum powder				
1.	10	0(0)	0(0)	0(0)
2.	10	0(0)	0(0)	1(10)
3.	10	0(0)	0(0)	0(0)
4.	10	0(0)	0(0)	0(0)
5.	10	1(10)	0(0)	1(10
6.	10	0(0)	0(0)	0(0)
Total	60	1(1.6)	0(0)	2(3.3)
B. Body lotions				
1.	10	1(10)	0(0)	0(0)
2.	10	0(0)	0(0)	0(0)
3.	10	0(0)	0(0)	0(0)
4.	10	0(0)	0(0)	0(0)
Total	40	1(1.6)	0(0)	0(0)

Table-3: Distribution of Hazardous Bacterial in tested preparations.

Discussion:

The results of study showed that talcum powders were generally more heavily contaminated than body lotions. From random selection of items it's not possible to determine whether the observed contamination reflects poor manufacturing conditions, post process contamination or over long storage by the retailer. For those products where high colony counts were observed also in the repeat examinations it is probable that the high coding counts reflect poor manufacturing conditions. Tests to assess whether these products might have become spoiled by growth of the contaminating organisms were not undertaken, but other worker [3] have demonstrated that deterioration can occur in products which are inadequately preserved. The incidence of significantly contaminated samples in the present investigation is similar to the levels previously reported by wolven and levenstein[15] reported an incidence of contamination of 24.4% (61 out of 250 items) whilst Dunnigan and Evans [16] observed contamination in 33 (19.5%) out of 165 items of cosmetic examined. Wolven and levenstein [17] have, shown a much lower incidence of contamination in the U.S.A, only eight (3.5%) of 223 items examined being contaminated. More recently Ashour and Abdelaziz [18] have shown a much higher incidence of contamination in Egypt

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(viable bacterial counts above 100 CFU/gm were recovered from more than 70% and less than 40% of the tooth pastes and mouth washes, respectively.

In another study ashour[19] have shown that talcum powders were more heavily contaminated with bacteria and fungi than body lotions (30% of talcum powders were contaminated with 10^{14} C.F.U/gm and none of the body lotions were contaminated to that extent). In particular, awareness of the need to ensure good microbiological quality in raw materials, especially natural pigments and fillers, will have had an effect on the levels of microorganisms present in many products.

In devising any code of good manufacturing practice the absence of specific pathogens must be considered in addition to control of the over all level of contamination of the product. When complete product sterility is not feasible, cosmetic and toiletry preparations should be free from viable pathogens. Such as Pseudomonas aeroginosa, Salmonellae, Escherichia coli, Staphylococcas aureus and certain Clostridia. Raw materials of mineral origin, such as talc, may be contaminated with spores of soil clostridia induding Cl. tetan: [20] and Cl. perfringes. Whilst Cl. tetani contamination of talc is known to have caused at least one out break of tetanus in babies[20]. The significance of Cl. perfringens spores is less clear. Strains of Cl. perfringens are known to cause gas gangrene in man and animals, the route of entry to the body tissues being via wounds and abrasions in the skin[21]. However, the minimum infection dose of Cl. perfringens strains is probably considerably above the level at which any area of skin would become contaminated by a cosmetic preparation containing a relatively low number of spores per gram.

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