

MICROBIOLOGICAL QUALITY OF DIFFERENT BRANDS OF TALCUM POWDER IN INDIA**Sanchita Choubey*, Aboli Kulkarni, Komal Awale and Suchitra Godbole**

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ABSTARCT

Commercial cosmetic products have been reported to be responsible for serious skin infections, which were often ignored as the sources or vehicles of transmission of microorganisms. Cosmetic products need not be sterile but may contain low levels of microbial load during use. This study was conducted to determine and compare the level and type of microbial contaminants in commercial cosmetic products sold in the market. Five brands of commercially available cosmetic talcum powders were purchased from the markets of Pune city. The bacterial load in different commercially available talcum powders was evaluated. A serial dilution technique was carried out and plating was done by using standard spread plate technique. The efficacy of preservatives was evaluated by challenging the talcum powder. High bacterial load was observed in all the samples tested; all the products were found to be contaminated to varying degrees. This may be due to poor manufacturing process, poor hygiene, and contaminated raw materials. It is concluded that poor microbiological quality of the preparations investigated can be attributed to either a problem inherent in the formulation of this brands and / or poor manufacturing hygiene. It is hoped that the implementation of good manufacturing practice in the cosmetic industries will improve the microbiological quality of these products.

KEY WORDS: Talcum powder, Cosmetics, Bacteria, Microbiology, Safety testing, Formulation.**INTRODUCTION**

Talcum Powder is soft, white powder used in cosmetics, baby powders and personal hygiene products. Talcum powder is a seemingly harmless over-the-counter product used by many consumers to prevent rashes and keep skin free of moisture. As talcum powder is applied, talc particles become airborne and, when inhaled, can cause coughing, wheezing and, in some cases, a condition called talcosis, characterized by acute or chronic lung irritation. Talc-based powders marketed as body powders or feminine hygiene products have also been linked to an increased risk of ovarian cancer and other major side effects in women, caused by the talc particles traveling through the vagina, into the uterus and along the fallopian tubes to the ovaries. The field of cosmetics and microbiology had not come into contact much before the 1930s and cosmetic microbiology became more important in 1940s.^[1] Talcum powders have positive effects on adult and babies skin, which mainly used to smooth the appearance of skin but negative effects also occur if they were contaminated. It was reported that some of these talcum powders are contaminated with spores of microorganism and can support their growth when they are poorly preserved and causes several disease.^[2]

The warm and rather humid climatic conditions would tend to support the survival and growth of many microorganism, so rapid growth and multiplication would be expected. This could lead to biodegradation of the product and hence the risk of microbial contamination to consumers of the product.^[3]

Antimicrobial Preservatives are substances added to dosage forms to protect them from microbial contamination. However, in many cosmetics no expiry date has been reported and may lose the Preservative activity and thus they become a potential risk for microbial contamination. Two different problems arise when preservatives are used in cosmetics, first is that microorganisms easily contaminate the cosmetics when the amount of antimicrobial agents are kept low for safety and economy, and second is that serious problems of skin reactions produced by antimicrobial agents are caused when their amounts are increased for preventing microbial contamination, therefore a balance needs to be established with the preservatives of choice between killing microorganisms and not injuring the cell of the consumer who uses the product. There are several different preservatives available but the cosmetic market is dominated by a few preservatives: paraben,

formaldehyde, and methyl chlorisothiazolinone / methylisothiazolinone.^[4] The Food and Drug Administration expressed its concern about in-use preservative adequacy of cosmetics in a Federal Register notice in 1977. This notice stated that regulatory action would be taken "to remove from the market any cosmetic that poses an unreasonable risk of injury because of inadequate preservation to withstand contamination under customary conditions of use".^[5] As early as 1970, cosmetic trade associations and individual companies recommended that consideration be given to continued effectiveness of a cosmetic's preservative system under intended consumer use conditions.^[6,7,8] More recently, consumer test programs to assess in-use preservative adequacy have been described.^[9]

According to The Federal Food and Drug Cosmetic Act criteria, cosmetic means the articles intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body or any part for cleansing, beautifying, promoting attractiveness, or altering the appearance. Contamination of microorganisms in cosmetics may cause spoilage of the product and when pathogenic, they represent a serious health risk for consumers.

The microbial contamination of personal care products may occur already in the course of production, through raw materials, ingredients and handling, or the contamination of a final product may ensue through its repeated use by the consumer. Different dangerous bacterial and fungal genera were found in cosmetic samples. The need to control microbiological contamination of products has been of considerable concern to cosmetic manufacturer.

The objective of this study was to assess the microbial quality of some selected brands of commonly used adult and baby powders to recommend the possibility of some health risk to consumers. To overcome this problem the packaging of talcum powder must be done in aseptic condition and dry places, and machinery used for grinding must be free from microbial contamination and to avoid the skin problem caused by the contaminated talcum powder, antibiotic must be used.^[10]

MATERIAL AND METHODS

Sample collection

A total of 5 commercial samples of talcum powder brands were purchased from the market of Pune city. For precaution, the seal of products were checked and transported to the laboratory and analyzed. The samples were stored at 4°C until microbial analysis was performed.

Evaluation test

10 gm samples were aseptically suspended in 100ml of nutrient broth medium and shaken for 10 minutes at room temperature, and incubated for 24-48 hours at 37°C. After incubation, samples were serially diluted up

to 10⁻⁴. Dilutions were spread on sterile Nutrient agar (NA) plates. The plates were allowed to solidify and incubated at 37°C for 24-48 h.

Identification of bacterial isolates

After incubation the isolated colonies were obtained in pure culture and identified up to species level. All bacterial isolates were identified based on their colony characteristics, morphological characteristics and biochemical reactions.

Detection of pathogens

According to the National Organization for Drugs Control and Research samples were tested for the pathogens mainly *E. Coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*.

Ten gram of cosmetic samples were aseptically suspended in 100 ml of lactose broth medium, and shaken well for 15 min at room temperature, and incubated for 24h at 37 °C ±2. After incubation, loopful of the original suspensions were streaked on Mac Conkey agar, and triple sugar iron agar tubes media to detect *E. coli*. Moreover loopful of suspensions were streaked on plates of mannitol salt agar (MSA) for detection of *Staphylococcus aureus*. The plates of cetrimide agar medium isolation agar media were used to detect *Pseudomonas aeruginosa*. The bacterial isolates were picked up, purified and sub cultured for further identification.^[11]

Efficacy test for preservative

Antimicrobial preservatives are substances added to products to protect them from microbiological growth or from microorganisms that are introduced inadvertently during or subsequent to the manufacturing process. In the case of products packaged in multiple dose containers, antimicrobial preservatives are added to inhibit the growth of microorganisms that may be introduced from repeatedly withdrawing individual used doses. Upon determination that a product has been properly utilized and has very low level of contamination, the user can conduct the preservative Efficacy test. The test is required for all cosmetic and personal care products in United States and follows the guidelines set forth by USP51.

Product found to be free of microbial contamination were challenged with *P. aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739. Inoculae for this test were prepared by harvesting colonies of the test organism grown on Nutrient agar with sterile phosphate buffer pH 7. This suspension contained 2 × 10⁸ CFU ml⁻¹ of *Pseudomonas* and 2 × 10⁸ CFU ml⁻¹ of *Escherichia coli* as determined by plate count. The challenge test procedure involved the inoculation of 1 ml test suspension into 50ml sterile water contains 10gm of talcum powder. Challenges were made with mixed cultures. The samples were spread on sterile nutrient agar plates, then were incubated at 30 to 35° C ± 2 for 5 days prior to counting;

the number of CFU/ml in each sample was recorded. Microbial content of each product was assayed at 0, 7, 14, 21, and 28 days.

RESULTS AND DISCUSSION

Cosmetics can be contaminated with microorganisms when they are not preserved properly. Contamination of microorganism in cosmetics may cause spoilage of the product and if pathogenic organisms have been detected, they represent a serious health risk. Most of the cosmetics are not sterile and they are made of non-sterile raw material. Although cosmetics do not have to be sterile, limit values have been reported according to the type of the cosmetics.^[12]

A total of 5 commercial samples of talcum powder brands were purchased from the market of Pune city, denoted as Sample-1, Sample-2, Sample-3, Sample-4, and Sample-5. Microorganisms were isolated from cosmetic samples. The isolated cultures were identified and confirmed by studying Evaluation test, morphological characteristics, and biochemical characteristics, the results obtained show that the bacterial load of talcum powder ranges from 86×10^4 to 187×10^4 CFU/g (Table 1).

Table 1: Enumeration of bacteria from talcum powder.

Sample No.	Dilution factor	No. of colonies	CFU/g
1	10^{-4}	86	86×10^4
2	10^{-4}	187	187×10^4
3	10^{-4}	62	62×10^4
4	10^{-4}	184	184×10^4
5	10^{-4}	108	108×10^4

The international Microbiological standards recommended limits for bacterial contamination in cosmetic products are 1.0×10^3 CFU/g for bacteria. High bacterial load observed in the present study could be due to poor manufacturing practice and improper storage. Identification of isolates obtained from samples was done by morphological characterization, microscopically by Gram staining, and various biochemical tests recommended in the Bergey's Manual of Determinative Bacteriology (Table 2).^[13,14] According to the tests, the organisms in sample-1 may be Citrobacter, sample-2 and 4 may be Staphylococcus, sample-3 and 5 may be Enterobacter (Table 2).

Table 2: Morphological, Biochemical and Physiological Characterization of the Microorganism
2 (a) Colony & Morphological characteristics

Characteristics	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Shape	Circular	Circular	Circular	Circular	Circular
Colour	Colorless	Golden-yellow	White	Golden-yellow	White
Margin	Regular	Regular	Regular	Regular	Regular
Opacity	Opaque	Opaque	Opaque	Opaque	Opaque
Elevation	Convex	Flat	Convex	Flat	Convex
Consistency	Smooth	Smooth	Smooth	Smooth	Smooth
Gram character	Gram negative short rod	Gram positive Cocci	Gram negative straight rod	Gram positive Cocci	Gram negative straight rod
Motility	Motile	Non-motile	Non-motile	Non-motile	Non-motile

2 (b) Biochemical tests for the isolates.

Biochemical test	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Sugar fermentation					
Glucose	G	A	A	A	A
Maltose	A	A	A	A	A
Mannitol	A	A	A	A	A
Lactose	A	A	A	A	A
Oxidase	-ve	-ve	-ve	-ve	-ve
Catalase	+ve	+ve	+ve	+ve	+ve
IMViC					
Indole	-ve	-ve	-ve	-ve	-ve
Methyl red	+ve	+ve	-ve	+ve	-ve
Voges Proskauer	-ve	+ve	+ve	+ve	+ve
Citrate Utilization	+ve	+ve	+ve	+ve	+ve
Nitrate reductase	-ve	+ve	+ve	+ve	+ve
Gelatinase	-ve	-ve	-ve	-ve	-ve
Isolated bacterial species	Citrobacter	Staphylococcus	Enterobacter	Staphylococcus	Enterobacter

-ve=Negative, +ve=Positive A=Acid, G=Gas

The results obtained by other investigators showed that all the tested cosmetic samples were free of Salmonella species, as the presence of Salmonella in the cosmetic products may be very rare Staphylococcus aureus, Pseudomonas aeruginosa and E. coli are the common pathogenic bacteria found in cosmetic samples.^[15,16] In

the present study Staphylococcus aureus pathogenic bacteria were present in cosmetic samples 2 and 4 (Table 3), all the tested cosmetic samples were free of E.coli and Pseudomonas pathogenic bacteria.

Table 3: Detection of Pathogens in Samples.

Sample No.	E.coli		Staphylococcus aureus	Pseudomonas
	MacConkey's Agar (Brick- red colonies)	Triple Sugar – Iron agar slant (Yellow + gas)	Mannitol Salt agar (Yellow colonies with yellow zone)	Cetrimide agar (Greenish fluorescence colonies)
1	-ve	-ve	-ve	-ve
2	-ve	-ve	+ve	-ve
3	-ve	-ve	-ve	-ve
4	-ve	-ve	+ve	-ve
5	-ve	-ve	-ve	-ve

-ve=Negative +ve=Positive

Challenge test for Preservative capacity was performed for the products that were not contaminated, using P. aeruginosa ATCC 9027, Escherichia coli ATCC 8739. Based on the bacterial challenge testing results, the

products were classified as poorly preserved, marginally preserved or well preserved. Results are shown in Table 4 and 5 and Fig. 1 & Fig. 2.

Table 4: Preservative Efficacy test against E. coli.

Sample	CFU/g of product at day post challenge					Interpretation
	Initial day	7 th day	14 th day	21 th day	28 th day	
1	51	244	TNTC	TNTC	TNTC	Preservatives are not effective
2	63	332	TNTC	TNTC	TNTC	Preservatives are not effective
3	68	372	TNTC	TNTC	TNTC	Preservatives are not effective
4	81	360	TNTC	TNTC	TNTC	Preservatives are not effective
5	98	TNTC	TNTC	TNTC	TNTC	Preservatives are not effective

TNTC- Too numerous to count.

Table 5: Preservative Efficacy test against Pseudomonas.

Sample	CFU/g of product at day post challenge					Interpretation
	Initial day	7 th day	14 th day	21 th day	28 th day	
1	38	40	212	TNTC	TNTC	Preservatives are not effective
2	24	33	256	TNTC	TNTC	Preservatives are not effective
3	61	78	TNTC	TNTC	TNTC	Preservatives are not effective
4	53	59	284	TNTC	TNTC	Preservatives are not effective
5	50	61	276	TNTC	TNTC	Preservatives are not effective

TNTC- Too numerous to count.

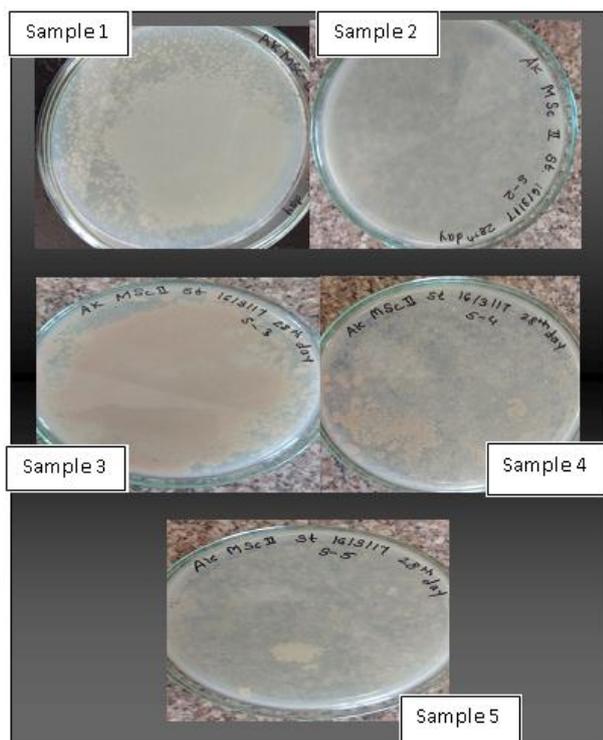


Fig. 1: Efficacy test 28th days of *E. coli*.

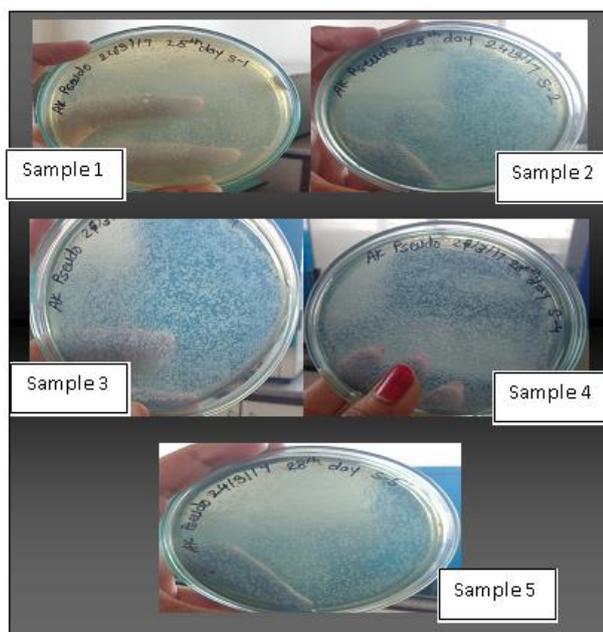


Fig. 2: Efficacy test 28th days of *Pseudomonas*

The results presented in this study show that the preservatives employed in the cosmetic products did not possibly possess adequate preservative capacity to be able to bring about acceptable low levels of microbial contamination, as demanded by regulatory bodies. Microbial challenge testing of cosmetics typically includes organisms resistant to preservatives.^[17] Resistant organisms are common and well known in the trade.^[18,19,20,21]

CONCLUSION

The results obtained in this study shows that all the five samples of talcum powder are capable of causing health hazards due to high microbial loads. This may be due to poor manufacturing practices, poor hygiene, contaminated raw materials or the susceptibility of the ingredients contained in the talcum powder.

Cosmetics can be contaminated with microorganisms when they are not preserved properly. Contamination of microorganism in cosmetics may cause spoilage of the product and if pathogenic microorganisms are present, they represent a serious health risk. Most of the cosmetics are not sterile and they are made of non-sterile raw material. Although cosmetics do not have to be sterile, limit values have been reported according to the type of the cosmetics. The presences of organisms such as *Citrobacter*, *Staphylococcus*, *Enterobacter* in the talcum powder samples implies that they can serve as vehicles for the transmission of disease. The microbial contamination of personal care products may occur already in the course of production, through raw materials, ingredients and handling, or the contamination of final product may ensue through its repeated use by the consumer. The need to control microbiological contamination of the products has been of considerable concern to cosmetic manufacturer. High microbial load was observed in the study could be caused by poor manufacturing practice and improper storage. The frequency of occurrence of bacteria in the samples shows that all the samples are contaminated with bacteria which indicating that talcum powders can permit the growth of bacteria.

Contaminating microorganisms in cosmetic may cause spoilage of the product and represent a serious health risk for consumers. Therefore, the need to control microbiological contamination of products has been of considerable concern to manufacturer. Modern pharmaceuticals, cosmetics and toiletries strive for high microbiological standards to protect their products from spoilage on the hand, and their consumers from infection, on the other hand unlike foodstuffs, which are usually kept refrigerated (or thrown away after a few days); a much longer shelf life is expected of personal care products.^[22] The microbial contamination of personal care products may occur already in the course of production, through raw materials, ingredients and handling, or the contamination of a final product may ensue through its repeated use by the consumer. Different dangerous bacterial and fungal genera were found in cosmetic samples. Therefore, Good Manufacturing Practice (GMP) should be strengthened, the efficacy, and continued use of the adopted preservatives should be reviewed, to ensure wholesomeness of the products through their shelf life.^[23]

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