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A survey of microbiological contamination in cosmetics and toiletries in the U.K. (1971)

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Synopsis—One hundred and seventy-two toiletry and cosmetic items purchased in 1971 from retail outlets throughout England and Wales were examined microbiologically. Viable MICRO-ORGANISMS were not recovered from over 50% of the items tested and about 90% contained fewer than 1000 organisms g^{-1}. 75% of all POWDER PREPARATIONS tested did not contain viable SPORES of ANAEROBIC BACTERIA and none contained more than 300 spores of anaerobic bacteria g^{-1}. Of the anaerobes isolated, none was identified as *Clostridium tetani*. *Coliform bacteria* were not detected in any preparation of toothpaste or lipstick examined. Comparison of counts from the top and bottom ends of metal foil tubed products showed almost identical counts in most cases, but in two instances significantly higher counts were observed in the top (nozzle end) sample. Further analyses were performed on six or twelve replicate items of a single brand of seven product types to check the inter-sample variation in count. The results obtained confirmed the overall level of colony count observed previously for these products; in some instances marked inter-sample variation in count was seen.

INTRODUCTION

Although many cosmetic, toiletry and pharmaceutical preparations contain preservatives (1–4) microbiological spoilage can still occur (5, 6).

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In some instances microorganisms in cosmetic preparations, whether present initially or transferred to the product by the user, have been implicated as the aetiological agents of disease. Wilson and co-workers (7, 8) have demonstrated that eye cosmetics may serve as a possible vector in transmission and persistence of microorganisms in clinical infections of the eye. An outbreak of tetanus in babies has been attributed to the use of talcum powder contaminated with *Clostridium tetani* (9). Other examples of the contamination of non-sterile drugs and cosmetics are given by Bruch (10, 11).

In 1967 the Toilet Preparation Federation and the Society of Cosmetic Chemists of Great Britain established a Select Committee to report on matters relating *inter alia* to the quality and safety of cosmetic preparations. One aspect of the work of this committee was to advise on the desirability for the establishment of microbiological standards for such products (12). Although many manufacturers have data on the microbiological quality of products immediately post manufacture, such information is not generally available. Furthermore, it may bear only a superficial relationship to the microbial quality of the products as purchased by the user. The present investigation was undertaken in 1971 at the instigation of the Select Committee to assess the incidence of contamination in a restricted range of cosmetic and toiletry preparations on sale to the general public.

Materials and methods

Provision of cosmetics

Products were purchased by representatives of the Toilet Preparations Federation in six areas of England and Wales. Two units (one of large size and one of small size whenever possible) were taken for each product from a large and a small retail outlet respectively. In some cases, additional items were provided for analysis. In total 172 cosmetic items were examined. Further items of selected products were purchased locally to investigate the inter-sample variation in counts.

Sampling of products

The outside surfaces of all containers were swabbed with 70% v/v ethanol before opening.

In general, 1 g samples were taken for each product, but in some instances 10 g or 0.1 g samples were examined. The sampling procedures used were as follows.

Talcum powder

An adequate quantity of the powder was shaken into a sterile petri dish, mixed and a representative sample was weighed into a tared bottle.

Loose and compressed face powder

After aseptically removing the seal, a sample of powder was scraped from the entire surface of the product into a sterile petri dish. For mixed samples the complete contents of the container were ground using a sterile pestle and mortar.

Complete make-up

Bottled products were mixed by inversion 20 times through an arc of 1 ft and a representative sample was removed using a wide-bore sterile pipette. For tubed products a large sample was extruded into a sterile bottle, mixed thoroughly and a sample for analysis was taken with a sterile spatula.

Face and hand creams, cake mascara and eye shadow

Products were sampled by taking a surface scrape as detailed above for face powder.

Liquid products (shampoo, bath oils, eye shadow)

The samples were mixed by inversion and an aliquot was removed by pipette.

Toothpaste and other tubed products

Samples were removed aseptically by extrusion through the nozzle. A second sample of each product was obtained by aseptically removing the crimped end of the tube and extruding a suitable sample.

Soap cakes

These were scraped with a sterile scalpel to remove wafer thin shavings from the entire surface. The shavings were mixed and a representative sample was taken for analysis.

Aerosol shaving soap products

These were voided into sterile wide neck jars. The sample was mixed and a 1 g aliquot weighed into a sterile wide neck bottle. After addition of 9 ml diluent, a few drops of sterile Antifoam A was added and the contents were mixed by swirling.

Lipstick

The whole or half surface of a lipstick was sampled by swabbing with a calcium alginate wool swab which was then transferred to sterile Calgon–Lubrol broth (see *Table I*) to effect solution of the alginate and dispersal of the lipstick 'fat'.

Table I. Diluents used in the analyses of cosmetics and toiletries

Sample type	Examination	Diluent	Footnote
Talcum and face powders	Aerobic counts	Tween-Peptone	1
	Anaerobic counts	Tween-RCM or RCM	2
Water-based creams and emulsions	Aerobic counts	Tween-Peptone	1
Eye cosmetics			
Toothpastes			
Bath oil, detergents	Aerobic counts	Peptone	3
Shampoo, soap			
Lipstick	Aerobic counts	Calgon-Ringer-Lubrol	4
Oil-based creams and emulsions	Aerobic counts	Lubrol broth	5

Composition of diluents

(1) Tween-Peptone: 0.1% w/v Peptone solution (pH 7.0) containing 0.1% v/v Tween 80.
(2) Tween-RCM: Reinforced Clostridial Medium (Oxoid), containing 0.1% v/v Tween 80. RCM: Reinforced Clostridial Medium (Oxoid).
(3) Peptone: 0.1% w/v Peptone solution, pH 7.0.
(4) Calgon-Ringer-Lubrol: 1 tablet of Calgon-Ringer (Oxoid) dissolved in 6 ml distilled water plus 4 ml 4% w/v Lubrol W solution.
(5) Lubrol Broth: 4 ml 4% w/v Lubrol W solution plus 5 ml Nutrient Broth (Oxoid)—see Ref. (2).

Microbiological procedures

In general the methods used were those recommended by Van Abbé *et al.* (2). Ten-fold serial dilutions were prepared in an appropriate diluent (*Table I*). Aerobic bacterial colony counts were made by pour plate technique on plate count agar (PCA; Oxoid). Plates were incubated in duplicate at 30°C for 3 days and at 37°C for 2 days. In later experiments counts of

bacteria were made at 25°C for 5 days instead of at 30°C for 3 days. Counts of yeasts and moulds were made on Sabouraud Dextrose Agar (SDA; Oxoid) incubated for 5 days at 25°C.

Counts of anaerobic sporeforming bacteria were made on Reinforced Clostrial Agar (RCA; Oxoid) after pasteurization (30 min at 75°C) of dilutions made up in freshly steamed Reinforced Clostridial Medium (Oxoid). The plates were incubated for 3 days at 37°C in an atmosphere of 95% hydrogen plus 5% CO_2. Clostridia were identified by the methods described by Willis (13). Dilutions of lipstick and toothpaste samples were tested for the presence of presumptive coliforms by inoculation into MacConkey Bile Salt Broth (Oxoid) which was incubated at 37°C and examined after 1 and 2 days.

After incubation the number of colonies was recorded for each plate. Arithmetic mean counts were derived for each item from those plates having from 30 to 300 colonies. In the case of samples with low counts the number of colonies recorded on the first dilution tested was used to derive the count. In some instances, the presence of an antimicrobial agent in the product was shown by a carryover effect where the count at high dilution was sometimes greater than the count at low dilution. Repeat analyses were always undertaken in such cases.

Mould growth on compressed eye make-up

The remainder of each sample after surface scraping was aseptically dissected into three portions, each of which was placed on a moistened sterile filter paper in a petri dish. The surface of the make-up was moistened with sterile water and the samples were incubated in a moist atmosphere for several weeks at 25°C; sterile water was added as necessary to the samples.

Statistical analysis

When replicate samples were tested, the significance of the difference in mean count for samples from different sources (e.g. surface scrape or mixed sample) or for replicate plates incubated at different temperatures was tested using two methods. For those results where large numbers of 'sterile' items (i.e. < 10 cfu g^{-1}) were obtained, Wilcoxson's Signed Ranks test was used (14). Where mainly definitive results were obtained, the mean difference of the $\log_{10}$ counts from zero was tested using Student's t. Counts of $<10\ g^{-1}$ were in all cases assigned the numerical value of 10 in calculations of the t value.

RESULTS

A total of 190 analyses were carried out on the 172 items purchased during the survey. The additional 18 tests were conducted to compare the counts on top and bottom samples from all tubed products, e.g. toothpaste, hair dressing, etc. A further 60 analyses were made on items of selected products purchased locally.

Aerobic bacteria

The distributions of aerobic bacterial colony counts at 30° and 37°C are summarized in *Tables II and III.* Over 80% of the items tested contained fewer than 300 cfu g^{-1}. Viable bacteria were not recovered from over 50% of the items examined. No difference was seen in the bacterial colony counts on samples from the top and bottom ends of 16 tubed products. Most samples yielded no viable bacteria from either end of the tube but in one case colony counts of 2.6×10^4 and 3.0×10^4 cfu g^{-1} were recorded for the top (nozzle end) sample at 30° and 37°C respectively whereas counts from the crimped end sample were 1.32×10^5 and 1.34×10^5 cfu g^{-1} respectively. In this instance general contamination of the product prior to filling may have occurred. In contrast, significantly different counts were noted in two items. The counts at 37°C on these items were 1.65×10^4 and 2.5×10^3 cfu g^{-1} for the top samples and 10 and 35 cfu g^{-1} respectively for the bottom samples. In these two instances it is probable that the nozzle end of the tubes was contaminated prior to filling with the product. Coliform bacteria were not detected in 0.1 g of any toothpaste sample nor on any lipstick sample examined. The predominant microorganisms isolated from high count products were Gram negative non-sporing rods, but no attempt was made to identify the organisms.

Anaerobic bacteria

Samples of each item of talcum powder, face powder and 'complete make-up' were examined for spores of mesophilic anaerobic bacteria. The distribution of counts is summarized in *Table IV.* Statistically significant counts (>300 cfu g^{-1}) were not obtained from any product examined in the survey, but such counts were later observed in the repeat analyses (see below). Selected colonies of anaerobes were subcultured and examined both microscopically and culturally. The organisms were typically mesophilic

Table II. Distribution of aerobic colony counts on PCA incubated for 3 days at 30° C

Product	No. of items	No. and (%) of items with colony counts g^{-1} within the range			
		< 300	300–1000	1001–10 000	>10 000
Powders					
Talcum powder	12	12 (100)	0 (0)	0 (0)	0 (0)
Face powder and rouge	14	12 (86)	0 (0)	2 (14)	0 (0)
Complete make-up	11	7 (64)	0 (0)	3 (27)	1 (9)
Creams and lotions					
Hand and body lotion	21	19 (90)	1 (5)	1 (5)	0 (0)
Face cream	17	12 (70)	2 (12)	1 (6)	2 (12)
Skin perfume	6	4 (67)	2 (33)	0 (0)	0 (0)
Hair cream and dressing	16	15 (94)	1 (6)	0 (0)	0 (0)
Shaving cream and foam	6	5 (83)	0 (0)	0 (0)	1 (17)
Eye make-up					
Mascara, eyeliner and eye shadow	20	13 (65)	3 (15)	1 (5)	3 (15)
Soaps and detergents					
Bath oil and detergent	18	16 (89)	0 (0)	1 (6)	1 (6)
Shampoo and hair colourant	13	12 (92)	0 (0)	1 (8)	0 (0)
Soap	6	6 (100)	0 (0)	0 (0)	0 (0)
Miscellaneous					
Toothpaste	6	6 (100)	0 (0)	0 (0)	0 (0)
Lipstick*	6	6 (100)	0 (0)	0 (0)	0 (0)
Total	172	145 (84)	9 (5)	10 (6)	8 (5)

* cfu/lipstick surface, not per g.

clostridia. *Cl. tetani* was not detected but 16 of the 50 isolates examined were identified as *Cl. perfringens.*

Yeasts and moulds

The distribution of counts of yeasts and moulds is presented in *Table V.* Of those items containing viable organisms, most were contaminated more heavily with yeasts than with moulds, the level of mould contamination rarely exceeding 50 cfu g^{-1}. Growth of moulds on moistened samples of cake mascara, rouge, and similar products did not occur during a three-month period at 25°C. On three occasions, heavy growth of bacteria occurred on plates of SDA. In each instance the bacteria were Gram negative rods, which also comprised the predominant flora of the items tested (liquid eye make-up). Such growth could have been avoided by the use of an antibiotic-containing medium (15).

Table III. Distribution of aerobic colony counts on PCA incubated for 2 days at 37° C

Product	No. of items	No. and (%) of items with colony counts g^{-1} within the range			
		< 300	300–1000	1001–10 000	>10 000
Powders					
Talcum powder	12	12 (100)	0 (0)	0 (0)	0 (0)
Face powder and rouge	14	12 (86)	0 (0)	2 (14)	0 (0)
Complete make-up	11	8 (73)	1 (9)	1 (9)	1 (9)
Creams and lotions					
Hand and body lotion	21	20 (95)	0 (0)	1 (5)	0 (0)
Face cream	17	16 (94)	0 (0)	0 (0)	1 (6)
Skin perfume	6	5 (83)	1 (17)	0 (0)	0 (0)
Hair cream and dressing	16	14 (87)	1 (6)	1 (6)	0 (0)
Shaving cream and foam	6	5 (83)	0 (0)	0 (0)	1 (17)
Eye make-up					
Mascara, eyeliner and eye shadow	20	13 (65)	4 (20)	2 (10)	1 (5)
Soaps and detergents					
Bath oil and detergent	18	17 (94)	0 (0)	1 (6)	0 (0)
Shampoo and hair colourant	13	12 (92)	0 (0)	1 (8)	0 (0)
Soap	6	6 (100)	0 (0)	0 (0)	0 (0)
Miscellaneous					
Toothpaste	6	5 (83)	0 (0)	1 (17)	0 (0)
Lipstick*	6	6 (100)	0 (0)	0 (0)	0 (0)
Total	172	151 (88)	7 (4)	10 (6)	4 (2)

* cfu/lipstick surface, not per g.

Table IV. Distribution of colony counts of clostridia from powders on RCA incubated for 3 days at 37°C

Product	No. of items	No. and (%) of items with colony counts g^{-1} within the range		
		< 10	10–300	>300
Talcum powder	12	8 (67)	4 (33)	0 (0)
Face powder and rouge	14	9 (65)	5 (35)	0 (0)
Complete make-up	11	11 (100)	0 (0)	0 (0)
Total	37	28 (75.6)	9 (24.4)	0 (0)

Table V. Distribution of colony counts for yeasts and moulds on SDA at 25°C

Product	No. of items	No. and (%) of items with colony counts g^{-1} within the range		
		< 300	300–1000	>1000
Powders				
Talcum powder	12	12 (100)	0 (0)	0 (0)
Face powder and rouge	14	12 (86)	1 (7)	1 (7)
Complete make-up	11	10 (91)	1 (9)	0 (0)
Creams and lotions				
Hand and body lotion	21	21 (100)	0 (0)	0 (0)
Face cream	17	17 (100)	0 (0)	0 (0)
Skin perfume	6	6 (100)	0 (0)	0 (0)
Hair cream and dressing	16	15 (94)	1 (6)	0 (0)
Shaving cream and foam	6	5 (83)	0 (0)	1 (17)
Eye make-up				
Mascara, eye liner and eye shadow	20	19 (95)	0 (0)	1 (5)
Soaps and detergents				
Bath oil and detergent	18	15 (83)	1 (6)	2 (11)
Shampoo and hair colourant	13	13 (100)	0 (0)	0 (0)
Soap	6	6 (100)	0 (0)	0 (0)
Miscellaneous				
Toothpaste	6	5 (83)	1 (17)	0 (0)
Lipstick*	6	6 (100)	0 (0)	0 (0)
Total	172	162 (94)	5 (3)	5 (3)

* cfu/lipstick surface, not per g.

Further analyses of selected items

Of the products examined during the survey, seven were selected for further investigation on the basis of the levels of contamination observed. Several units of each product were purchased locally from a number of different retail outlets. They were examined essentially as described above except that aerobic bacterial counts were made at 25°C rather than at 30°C and in some instances comparison was made of surface and mixed samples of the items. The distribution of counts is presented in *Table VI.*

The brand of liquid eye liner and bath detergent were selected because of the extremely high count observed previously in both items of each of these products. Further tests showed that the mean counts of the replicate items examined were also high (*c.* 10^5 cfu g^{-1}), but that the counts from different units ranged from no viable bacteria recovered to a count in one instance of 1.2×10^6 at 25°C. Significantly lower counts were observed at 37°C than at 25°C for these products.

Table VI. Replicate analyses of selected products

Product type	No. of items	Mean and (range) of colony forming units g^{-1} at		
		25°, PCA	37°, PCA	25°, SDA
Bath detergent	6	100 345 (< 10–205 000)	< 10	24Y < 10–80Y
Face cream				
Surface sample	6	10 (< 10–40)	31 (< 10–100)	NT*
Mixed sample	6	21 (< 10–45)	13 (< 10–35)	NT
Foundation cream	6	65 (< 10–210)	298 (< 10–700)	110Y, 65M† (< 10–320Y) (< 10–180M)
Toothpaste	6	< 10	10 (< 10–60)	< 10
Skin lotion	6	< 10 (< 10–10)	33 (< 10–95)	< 10
Eyeliner	12	121 758 (< 10–1 460 000)	41 (< 10–270)	27Y (< 10–160Y) 37° Anaerobic, RCA
Compressed face powder				
Surface sample	6	498 (280–660)	352 (215–470)	NT
Mixed sample	6	311 (90–550)	225 (85–390)	1180 (360–2700)

* NT, Not tested.
† Y=Yeasts; M=moulds.

Examination of replicate units of single brands of toothpaste, skin lotion/face cream and foundation cream for which counts below 300 cfu g^{-1} had previously been recorded, provided confirmatory evidence for the low levels of contamination of these items. The counts obtained from surface samples and mixed samples of the face cream did not differ markedly and were in any case below the level of statistical significance for the plate count method.

The replicate units of one brand of compressed face powder were also examined using both surface sample and mixed sample procedures. The counts obtained at 25° and 37°C were higher in most instances from surface samples than from mixed samples. However, although the difference was significant at the 5% level for counts at 37°C, no statistically significant

difference was observed for the counts made at 25°C. The counts of anaerobic bacteria on these units were much higher (mean count 1180 cfu g^{-1}) than had been observed previously.

Observations on packaging of the products studied

The majority of products were packaged such that post-packaging contamination would not be likely to occur before the product was used. Attempts to correlate colony counts with type of packaging were unsuccessful. Although a few products were clearly coded, this was not evident in the majority of items examined and the absence of coding would make difficult any retrospective attempt by the manufacturer to check against the production batch if consumer complaints subsequently occurred.

There were no obvious differences between colony counts from units of different size nor from small or large retail outlets. Although differences in brand gave rise to differences in the levels of contamination of particular product types it was not possible to determine the incidence of contamination for any particular brand because of the small number of items examined for individual brands.

Discussion

This survey demonstrates that in 1971 about 90% of a diverse range of cosmetic products and toiletry preparations contained fewer than 1000 viable microorganisms per g of product and that over 50% of the items examined were essentially 'sterile'. Of those items which were contaminated, few contained more than 10^5 organisms g^{-1}. The most heavily contaminated products were specific brands of eye make-up (especially liquid eyeliner), bath detergent and complete make-up. Unfortunately, tests for the presence of specific organisms such as *Pseudomonas aeroginosa* were not made on these products during the present investigation. From random selection of items it is not possible to determine whether the observed contamination reflects poor manufacturing conditions, post-process contamination or overlong storage by the retailer. For those products where high colony counts were observed also in the repeat examinations it is probable that the high colony counts reflect poor manufacturing conditions. Tests to assess whether these products might have become spoiled by growth of the contaminating organisms was not undertaken, but other workers (4, 5)

have demonstrated that deterioration can occur in products which are inadequately preserved.

The incidence of significantly contaminated samples (i.e. containing >300 cfu g^{-1}) in the present investigation is similar to the levels previously reported from the U.S.A. Wolven and Levenstein (16) reported an incidence of contamination of 24.4% (61 out of 250 items examined) whilst Dunnigan and Evans (17) observed contamination in 33 (19.5%) out of 165 items of cosmetic examined. Unfortunately, although the latter workers identified the predominant microflora they gave no information of the nature of the contaminated products. More recently, Wolven and Levenstein (18) have shown a much lower incidence of contamination of cosmetic products in the U.S.A., only eight (3.5%) of 223 items examined being contaminated. It is not unreasonable to suppose that similar improvements in the quality of cosmetics may have occurred during the past two years in the U.K. 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 (D. Spooner, personal communication).

In devising any 'Code of Good Manufacturing Practice' the absence of specific pathogens must be considered in addition to control of the overall level of microbial 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*, *Staphylococcus aureus* and certain clostridia. Raw materials of mineral origin, such as talc, may be contaminated with spores of soil clostridia including *Cl. tetani* (19) and *Cl. perfringens*. Whilst *Cl. tetani* contamination of talc is known to have caused at least one outbreak of tetanus in babies (9, 19) 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 (20). However, the minimum infective 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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