CHAPTER IV

DISCUSSION

In recent years increasing interest has been shown in the susceptibility of cosmetics and toiletry products to microbial contamination and the potential risk of infection to the user of those products (Baird, 1977).

There are now several requirements by control and official compendia for the microbial control of pharmaceutical and cosmetic preparations or raw materials. These include total counts and microbial limit tests for specific harmful organisms such as coliforms or *Escherichia coli*, *Salmonella*, *Pseudomonas* spp. and *Staphylococcus aureus* (Bowman, 1969; B.P.C., 1973; N.F., 1975; U.S.P., 1975). Some suggested standards are given in TABLE 18 (Davis, 1972).

TABLE 18

Tentative microbiological standards for cosmetic preparations g^{-1} or ml^{-1}

	Satisfactory	Doubtful	Unsatisfactory
Total colony count*	1000	1000-10000	10000
Presumptive coliforms*	10	10-100	100
Faecal coli	1	1-10	10
*Staph aureus**	1	1-10	10
*Ps. aeruginosa**	1	1-10	10
Salmonella		Not detectable in 100 g	
Fault producing organisms		Impossible to generalise	

* = first priority in laboratory control work.

Bruch (1971) has suggested other organisms which should be excluded from topical products and has ranked them in importance to the areas of body application. The organisms suggested are those mentioned previously, excluding Salmonellae, and with the addition of all other pseudomonads, *Klebsiella* and *Serratia marcescens* (Bruch, 1971).

For the estimation of the number of viable microorganisms, the count methods in common use are the pour-plate, roll-tube, surface, drop methods and the multiple-tube method or most probable number or index (MPN). Basically, these techniques consists of serially diluting the test sample, if necessary, in a suitable diluent and then adding aliquots of the dilution into or on suitable culture media.

Pour plate method is most widely used in microbiology and is now included in the United States Pharmacopoeia 19 th rev. (1975) for the microbial examination of products. It has also been used by many workers in various countries in their investigations on contamination of products: Denmark, Sweden, U.S.A., Great Britain, Belgium, Switzerland, Rumania and Czechoslovakia. Briefly, the method consists of mixing a measured amount of sample with molten agar medium in Petri dish and, after setting, incubating the inoculated dishes at various temperatures.

Many media are being used and the World Health Organization Study Group Report (WHO, 1960), listed nine media for culturing bacteria and six for culturing fungi. The groups could not recommend any one media in preference to another because of lack of comparative data. Since no single medium will support the growth of all bacteria, moulds and yeasts, so more than one medium should be used. The question of which to use has been, and still, is, the subject of many conferences and published reports. Pittman concluded that none of the reports have supplied adequate support for the selection of any medium in preference to others in use (Scott, 1973).

Eye cosmetics should have adequate preservative system to prevent proliferation of microorganisms which are transferred from the eye area to the cosmetic during use (McConville and Anderson, 1975). The CTFA recommended microbiological limits for eye cosmetic is 500 microorganisms per gram of product (Tenenbaum, S., 1972). Pseudomonads have

been implicated in corneal infection. A corneal abrasion could have occured from a tweezer, a cosmetic applicator or a finger nail possibly resulting in an infection (Gilardi, 1972; Marzulli, *et al.*, 1972). Fungal infections of the eyes are usually initiated by trauma, such as puncture of the cornea with a mascara brush or contamination during surgical procedures. Ulceration frequently develops around the site of trauma. Many different fungi can be etiological agents of eye infections. These include yeast such as *Candida albicans* and *Candida parapsilosis* as well as many other opportunistic fungi that are common soil saprophytes. Examples of some genera isolated from eye lesions include *Aspergillus* sp., *Cephalosporium* sp., *Cladosporium* sp., *Curvularia* sp., *Monosporium* sp., *Penicillium* sp., *Scopulariopsis* sp. etc. Many of these fungi enter topical drug and cosmetic products during manufacture and can only be removed by sterilization. Preservatives can prevent growth of these organism after the containers are intially used and contaminated by the consumer (Bruch, 1976). The important pathogenic bacteria, *Pseudomonas aeruginosa,* when grown in water, has an increased resistance to various antimicrobial materials such as chlorine dioxide, a quaternary ammonium compound, 0.25% acetic acid and activated glutaldehyde, than those same strains grown on trypticase soy agar. These facts could be interpreted to explain the observations that the pseudomonads encountered in a manufacturing environment can easily overcome the preservative system in a product (Favero, *et al.*, 1971).

Originally the reported contaminants of toiletries and cosmetics covered a wide spectrum of organisms (Wedderburn, 1964). The most

frequently reported contaminants in recent years have belonged to such genera as *Pseudomonas, Klebsiella, Achromobacter* and *Alcaligenes*. These bacteria are common residents in water and it is now widely believed that the water used in the preparation of toiletry products is their likely source (Malcolm and Woodroffe, 1975).

From the dermatological point of view, the most important requirement of talcum and other cosmetic powder is that the materials from which they are made should be free from harmful contaminants. It is desirable that all such materials should be subjected to bacteriological examination. Sterilization may be carried out by various means, both in respect of the raw materials and of the finished products (Davis, 1960). A number of workers have attempted to determine the incidence of contamination; the results from these surveys are conflicting and contamination rates have been found to vary from 2.5%-43% (Wolven and Levenstein, 1969; Dunnigan and Evans, 1970; Wilson, *et al.*, 1971; Wolven and Levenstein, 1972; Myers and Pasutto, 1973; Jarvis, *et al.*, 1974) One of the survey presented on 23 February 1976 at the Symposium on Microbiological Safety in Cosmetic and Toiletry Products, Birmingham, as shown in TABLE 19. Gram-negative rods were isolated from nine cosmetic products and *Pseudomonas aeruginosa* was isolated as shown in TABLE 20 (Baird 1977).

TABLE 19

Type of products sampled and number contaminated

Product	No.of Samples	No. contaminated	Type of contamination	number
Creams and Lotions				
Handcream	27	9	asb	4
			g+	5
			g-	1
Cleanser	24	12	asb	6
			g+	3
			g-	3
Foundation	8	5	asb	4
			g+	2
Medicated topical	8	2	asb	2
			g+	1
Eye make-up				
Shadow	5	5	asb	3
			g+	3
Liner	3	1	asb	1
Mascara	6	4	asb	1
			g+	2
			g-	1
Non-medicated eye drop	8	0	—	—
Baby				
Lotion and shampoo	12	1	g+	1
Dental				
Toothpaste	1	0	—	—
Denture cleaning solution	2	0	—	—
Denture powder and cream	2	2	g-	2
Teething gel	2	0	—	—

TABLE 19 (Continued)

Product	No.of Samples	No. contaminated	Type of contamination	number
Soaps and detergents				
Bath detergent	1	0	–	–
Shampoo	9	0	–	–
Conditioner	10	3	g+	1
			g-	2
Soap	1	1	asb	1
Miscellaneous				
Depilatory	6	1	g+	1
Deodorant	2	0	–	–
Sun-tanning and after-sun lotion	10	2	asb	1
			g+	1
Total	147	48(32.7%)	asb	23
			g+	20
			g-	9

Key, asb : aerobic spore bearer; g+ : non-haemolytic coagulase negative Gram-positive cocci; g- : Gram-negative rod.

TABLE 20

Gram-negative rods found in cosmetics

Contaminant	Product	No. of organisms (g^{-1} or ml^{-1})
Pseudomonas aeruginosa	Lanolin hand-cream	$1.2X10^3$
P. maltophilia	Mascara	$7.0X10^5$
P.pseudoalcaligens	Cleansing milk	$3.1X10^4$
P. pseudoalcaligenes	Hair cream	$1.9X10^4$
P. fluorescens	Hair oil	$4.0X10^4$
P. putida	Cleansing jelly	$2.5X10^4$
Moraxella osloensis	Moisture cream	$1.3X10^3$
Enterobacter cloacae	Dental cream	$2.3X10^5$
Klebsiella aerogenes	Dental powder	$3.4X10^6$
K. oxytoca	Dental powder	
Erwinia herbicola	Dental powder	
Enterobacter cloacae	Dental powder	

In this study the determination of microbial contamination in various kind of unused cosmetics produced domestically such as eye shadow, eye liner, powder lotion, shampoo and talcum powder revealed that 70 of 141 samples (50%) were contaminated with some bacteria and fungi. All of the pathogenic bacteria that should not be present in the cosmetic as recommended in the U.S.P. XIX were isolated from 18 samples (13%). *Escherichia coli* was isolated from 3 cosmetic products which include 1 eye liner and 2 shampoos. *Pseudomonas aeruginosa* was isolated from 7 cosmetic products which include 1 eye liner, 4 powder lotions and 2 shampoos. *Salmonella* species were isloated from 2 cosmetic products which include 1 eye-shadow and 1 shampoo. *Staphylococcus aureus* was isolated from 6 cosmetic products which include 1 eye liner, 1 eye shadow, 3 powder lotions and 1 shampoo. The other bacteria isolated was *Bacillus* species from 31 cosmetic products, 2 eye liners, 2 eye shadow, 4 powder lotions, 2 shampoos and 21 talcum powders. This means that the process of manufacturing is substandard and the talc grade used was low.

The fungi isolated were *Aspergillus, Penicillium, Curvularia* and *Paecilomyces*. *Aspergillus* was isolated from 25 cosmetic products, which include 3 eye make-ups, 3 powder lotions, 3 shampoos and 16 talcum powders. *Penicillium* was isolated from 4 talcum powders, *Paecilomyces* from 6 talcum powders and *Curvularia* from 1 powder lotion. None of these preparations showed visible signs of bacterial contamination or degradation, despite the fact that the counts ranged from $10\text{-}10^4$ colony gm^{-1} or ml^{-1}. It is disquieting to know that there still are

cosmetics in the market today which may inflict serious human injury. Lack of adequate protection against microbial contamination, during the manufacturing processess, is one of the important factor. Inadequate microbiological testing, product preservation, sanitary manufacturing procedures and self-regular efforts on the part of the cosmetic industry, are among other factors. Nowadays, there still are cosmetics in the market that contain pathogens or high population of other microorganisms that may be hazardous to consumers under debilitating conditions of use. In addition, little progress has been made to date by the industry in regard to the preservation of cosmetics during their use by customers. The accomplishment of adequate protection of cosmetics against microbial contamination at the time of use should now become the most important objective of cosmetic microbiologist (Idson, 1976). With one exception, the results from all surveys, including this one, are based on the examination of a limited number of samples, mostly less than 250 products. Interpretation of results, expressed as percentages, is therefore restricted, but these results may be used for comparative purposes.