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Microbial Flora of In-Use, Display Eye Shadow Testers and Bacterial Challenges of Unused Eye Shadows

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We surveyed 15 different brands of eye shadow on display for customer use in different retail stores for microbial contamination. This was the first reported microbial surveillance of in-use eye shadow display testers in retail establishments. Cultures were obtained at each retail store. Sterile dacron swabs were rolled and rubbed over the entire used surface of each shadow, and each inoculum was streaked onto the surfaces of blood agar plates. Of the 1,345 individual samples obtained, 67% were contaminated with one or more species of microorganisms representing the genera *Staphylococcus*, *Micrococcus*, *Corynebacterium*, *Acinetobacter*, *Bacillus*, and *Moraxella*. We also purchased two different brands of water-miscible eye shadows in replicate unit containers. Each brand was challenged separately with a few hundred to several thousand colony-forming units of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, or *Acinetobacter calcoaceticus*. Both brands permitted growth of *P. aeruginosa* but not growth of *S. aureus*. *A. calcoaceticus* was inhibited after inoculation into one brand. With the other brand, the inoculum of *Acinetobacter* multiplied in one of the two different lots tested. This experimental challenge procedure can serve as a useful model system for studying the behavior of microbes in eye shadows and similar matrices.

Studies of in-use mascaras that are applied to eye lashes have shown that these periocular cosmetics are often contaminated with microorganisms, which usually are representative of the normal human skin flora (3, 17, 30, 31). Eye shadows are another type of cosmetic applied around the eyes, usually on the upper lid. To the best of our knowledge, this was the first surveillance study of the type and incidence of microbial contamination associated with in-use store display eye shadow testers.

Eye shadows that are available for unrestricted use by customers are frequently placed on the counters of drug and department stores. These samples, called testers at cosmetic counters, are usually arranged in a plastic tray, with or without a cover. Each eye shadow is in a small metal cup or pan. The number of eye shadows in a tray varies with the brand. Occasionally, the testers are not assembled in rows in a tray, but are in individual plastic jars with lids (the same unit that can be purchased). Basic ingredients of eye shadows include talcs, kaolin, preservatives, coloring agents, and binders (22-24). Parabens and imadazolidinyl urea are commonly used preservatives. The color agents for eye shadows are frequently oxides of aluminum, iron, and chromium. Pearlescent shades contain one or more of the following: mica, bismuth oxychloride, $CaCO_3$, and certain other mineral compounds. Binders include cellulose and other natural gums, fatty alcohols, hydrocarbons, lanolin, and inorganic colloidal silicates. This diversity of organic and inorganic components in eye shadows could serve as nutritional substrates for the growth of certain types of microbes if the preservatives become inactivated, destroyed, or ineffective. We undertook a surveillance sampling study to assess the type and incidence of microbial contamination in various brands of in-use store display eye shadow testers.

Based on the recovery of microorganisms observed in this survey of eye shadows and from previous reports (1, 9, 12, 20, 21, 27) that suggested the importance of eye shadows in ocular infections, we chose three bacteria (*Acinetobacter calcoaceticus*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*) for in vitro challenge studies of store-purchased eye shadows in an attempt to determine the ability of these products to inhibit the growth of organisms that are introduced accidentally. These studies were designed so that they approximated the conditions of contaminated counter testers, including

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incubation temperature, approximate level of contamination, and time of contact between eye shadow and microorganisms.

MATERIALS AND METHODS

Samples. Swab samples were taken from eye shadow testers, tray sets, or individual jars. Approximately 2.0 to 4.5 mg of cosmetic was acquired by each swab sample. In-use testers that showed moderate to heavy usage were sampled in stores throughout the Atlanta, Ga., area during the regular business hours. All testers in each tray or series were sampled. Based on estimates obtained from store personnel, the display age of trays ranged from 6 to 24 months.

Sampling. A sterile dacron swab (Scientific Products) was rotated and rubbed over the surface of each cosmetic and then rotated and streaked onto the surfaces of 5% sheep blood agar plates (Columbia base or tryptic soy agar base; Difco Laboratories). Inoculated plates were returned to our laboratory within 1 to 2 h and were incubated for 48 h at 35°C.

Enumeration and characterization of colonies. After incubation, colonies were characterized according to size, gross morphology, presence or absence of hemolysis, and pigmentation. The number of colonies representing each distinctive type was recorded.

Identification of isolates. Smears from representative colonies were Gram stained. Microorganisms were identified by standard biochemical methods for gram-positive and gram-negative cocci and bacilli, as described in the *Manual of Clinical Microbiology*, 2nd ed. (18).

Eye shadow challenge studies. (i) Strains. The Bact-Chek (Roche Diagnostics) cultures of *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 9721 and a hospital strain of *A. calcoaceticus* subsp. *anitratus* were used.

(ii) Cosmetics. We purchased multiple samples of two brands of water-miscible, pressed-powder eye shadows (both blue shades) at a busy retail store. Each eye shadow was subdivided aseptically into portions of approximately 380 mg, which were added to sterile, capped, polypropylene test tubes (10 by 100 mm). Portions of each individual container of eye shadow were cultured on blood agar to detect aerobic microorganisms; none were found. The same lots of brand A and brand B were used for all challenges, with the exception of one repeat experiment with a second lot of brand A (the original lot was depleted), which was challenged with *Acinetobacter*.

Talcum (Fisher Scientific Co.), a basic component of dry, pressed-powder eye shadows, served as a control. The talcum was sterilized in a hot air oven for 2 h at 170°C and was apportioned into test tubes in the same manner and weight as the cosmetics.

(iii) Inocula. Each organism was grown on blood agar at 35°C for 24 h. One or more well-isolated colonies from blood agar were inoculated into 2.0 ml of normal saline to obtain slight turbidity. Inocula for the challenge tests were prepared by centrifuging these preparations and washing them three times with sterile saline; this was followed by serial dilution to attain three different concentrations for each organism.

A 0.1-ml amount of each dilution was inoculated into a test tube containing eye shadow. The resulting cultures were incubated at 25°C for 0 to 96 h. At different times (0 to 96 h) 0.9 ml of normal saline was added to each eye shadow, the powder-saline suspension was mixed vigorously, and 0.1-ml portions were withdrawn and inoculated onto tryptic soy agar (Difco) plates.

To accommodate expected increases in bacterial populations, additional serial dilutions of the powder-saline suspension were made and plated onto tryptic soy agar in duplicate.

RESULTS

A total of 67% of 1,345 testers yielded one or more microbial colonies (Table 1). Microorganisms were not recovered from 3 of the 15 brands, even though these 3 appeared to have had usage comparable to the contaminated brands. A total of 8,691 colonies were isolated from the positive testers (Table 1). Approximately 90% of all colonies represented common skin flora organisms (*Micrococcus* spp., *Corynebacterium* spp., *Staphylococcus epidermidis*, and *Bacillus* spp.). *S. aureus* and gram-negative rods were recovered occasionally, usually in low numbers. No pseudomonads were isolated during this study. The gram-negative rods that were recovered were *A. calcoaceticus* and *Moraxella* species. Molds were found frequently, but were almost always present as only one or two colonies.

Most microorganisms were recovered in amounts between 1 and 10 colony-forming units (CFU) per sample. However, *Micrococcus*, the most common organism isolated, yielded as many as 100 to 150 CFU per sample from two different samples.

The presence of *S. aureus* and gram-negative rods in some in-use testers stimulated interest in how sterile, previously unexposed, pressed-pow-

TABLE 1. *Frequency of isolation of microorganisms from a sampling of 1,345 eye shadow testers*

Microorganism	No. of positive wells	% Of positive wells	No. of colonies	% Of all colonies
Micrococcus spp.	524	40.0	3,564	41.0
Corynebacterium spp.	272	20.2	1,701	19.6
S. epidermidis	241	17.9	1,513	17.4
Bacillus spp.	419	31.2	1,033	11.9
Molds	228	17.0	646	7.4
S. aureus	31	2.3	87	1.0
Uncategorized bacteria	9	0.7	45	0.5
Actinomycetes	15	1.1	42	0.5
Gram-negative rods[a]	12	0.9	41	0.5
Yeasts	7	0.5	10	0.1
Neisseria spp.	8	0.6	9	0.1

[a] No members of the *Pseudomonadaceae* or the *Enterobacteriaceae* were isolated. *Acinetobacter* and *Moraxella* spp. were isolated.

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der eye shadows would respond to challenges with selected strains of bacteria. *S. aureus* and *A. calcoaceticus* were selected for study because of their recovery from the testers and their potential for causing diseases of orbital and periorbital tissues (1, 4, 12, 21, 27). Although *P. aeruginosa* was not isolated from the testers, this organism was also studied because of its obvious importance as a human pathogen and its role in serious infections of eyes (9, 11, 20).

The challenge experiments were designed to approximate possible levels of contamination. Measured inocula were introduced into the test powders. Dilution and plating of some of the initial inocula within a few minutes after introduction into the powders provided zero-time controls. The data indicated that, generally, excellent quantitative recovery was possible and that there was no obvious or immediate killing (Tables 2 through 5).

A popular pressed-powder eye shadow lacking protein (brand A) and another brand containing protein (brand B) were chosen for these tests.

TABLE 2. *Population changes when different S. aureus inocula were added to two different eye shadows and talcum*

Eye shadow	Inoculum (CFU/tube)	Recovery (CFU/0.1 ml) at:					
		Zero time	4 h[a]	24 h	48 h	72 h	96 h
Brand A	6,000	800	770	640	230	60	9
	600	40	20	20	0	0	0
	60	3	1	1	0	0	0
Brand B	6,000	510	670	720	60	2	0
	600	17	17	18	<1	0	0
	60	1	2	1	0	0	0
Talcum	23,000	2,000	1,520	1,120	110	30	5
	2,300	230	400	140	30	7	2
	230	60	50	20	2	2	2

[a] Length of time that cultures were incubated in cosmetic.

In the first challenge study, *S. aureus* was inoculated into the two brands, and the resulting cultures were incubated for up to 96 h (Table 2). A distinct decrease in CFUs was observed in samples plated after 24 h. By 96 h, all dilutions plated were negative, except for the heavily inoculated (6,000 CFU) cultures (99% decrease). A repeat challenge experiment yielded the same results. Cultures of *S. aureus* inoculated into talcum followed a pattern similar to the patterns of *S. aureus* cultures in the two cosmetics (Table 2).

The behavior of *P. aeruginosa* in brands A and B differed markedly from that of *S. aureus*. Increases in CFUs compared with the original inoculum size were found after 4 h of incubation in both brands. Platings from subsequent incubations showed significant increases in CFUs (Table 3). After 96 h of incubation, the two largest inocula in brand A yielded 1×10^6 to 1.6×10^6 CFU/0.1 ml. Large residual populations of *P. aeruginosa* were found 14 days after challenge in the two different cosmetics. The same patterns were observed in a repeat challenge. The multiplication of *P. aeruginosa* in talcum appeared similar to that observed in the cosmetics (Table 3).

A. calcoaceticus subsp. *anitratus* (Tables 4 and 5) did not grow and multiply in brand B. Cultures of *Acinetobacter* introduced into brand A lot 1 showed decreases in CFUs for the first 24 h with all inocula. However, the cultures which received the largest inoculum (5,500 CFUs) in brand A showed increases in CFUs between 24 to 96 h. Bacteria were not recovered at 72 and 96 h from the cultures which received the two smaller inocula. Because of the unavailability of the original lot in local stores, a different lot of brand A was used for the second

TABLE 3. *Population changes when different P. aeruginosa inocula were added to two different eye shadows and talcum*

Eye shadow	Inoculum (CFU/tube)	Recovery (CFU/0.1 ml) at:						
		Zero time	4 h[a]	24 h	48 h	72 h	96 h	14 days
Brand A	1,500	250	580	14,770	882,000	1.43×10^6	1.61×10^6	TNTC[b]
	660	40	100	TNTC	463,000	1.18×10^6	1.09×10^6	TNTC
	110	10	10	860	114,000	249,000	480,000	TNTC
Brand B	1,500	420	610	2,500	7,750	38,800	63,000	TNTC
	660	40	120	430	3,950	45,600	59,250	TNTC
	110	3	10	60	470	2,720	15,500	TNTC
Talcum	14,000	≅1,000	750	TNTC	728,000	1.95×10^6	2.05×10^6	
	1,400	370	90	830	25,200	362,000	840,000	
	300	30	20	260	19,000	383,000	510,000	

[a] Length of time that cultures were incubated in cosmetic.
[b] TNTC, Too numerous to count (usually 10^5 CFU/0.1 ml or more).

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TABLE 4. *Population changes when different A. calcoaceticus subsp. antitratus inocula were added to two different eye shadows and talcum*

Eye shadow	Inoculum (CFU/tube)	Recovery (CFU/0.1 ml) at:					
		Zero time	4 h[a]	24 h	48 h	72 h	96 h
Brand A (first lot)	5,500	360	310	10	60	130	3,530
	550	50	40	<1	2	0	0
	60	4	4	0	0	0	0
Brand B	5,500	270	200	10	<1	0	0
	550	30	20	1	0	0	0
	60	3	2	0	0	0	0
Talcum	10,000	600	1,700	22,670	251,000	400,000	530,000
	950	200	300	20,000	219,000	285,000	360,000
	300	30	20	880	77,000	99,000	290,000

[a] Length of time that cultures were incubated in cosmetic.

TABLE 5. *Population changes when different A. calcoaceticus subsp. anitratus inocula were added to two different eye shadows*

Eye shadow	Inoculum (CFU/tube)	Recovery (CFU/0.1 ml) at:					
		Zero time	24 h[a]	48 h	72 h	96 h	15 days
Brand A (second lot)	9,500	680	TNTC[b]	970,000	530,000	820,000	100,000
	1,050	80	12,720	237,000	670,000	300,000	209,000
	130	10	862	30,000	75,000	59,000	61,000
Brand B (control)	9,500	150	154	1	0	0	0
	1,050	50	20	0	0	0	0
	130	2	3	0	0	0	0

[a] Length of time that cultures were incubated in cosmetic.
[b] TNTC, Too numerous to count (usually 10^5 CFU/0.1 ml or more).

challenge. With this second lot, increases above the original inoculum sizes were observed after 24 h of incubation. *A. calcoaceticus* was recovered from the inoculated eye shadows at concentrations of 61,000 to 209,000 CFU/0.1 ml after 15 days of incubation. In talcum (Table 4) *Acinetobacter* increased in a manner comparable to that increase observed in brand A (Table 5).

DISCUSSION

The microorganisms recovered from the eye shadow display testers were mainly representative of the normal skin flora and probably some airborne contaminants (2, 19, 25). Gram-negative bacteria were rare contaminants.

An analysis of our data and our observations of women who used testers in stores suggested several probable modes of cosmetic contamination. Most contamination was probably introduced into the cosmetics by the frequent and common use of fingers and multiple-use applicators (foam-tipped swabs or brushes) to sample and spread the different eye shadows onto the eyelid. Applicators were never cleaned or disinfected by store personnel, and some applicators were worn out from overuse. Routine cultures of applicators usually showed moderate to heavy bacterial contamination. Applicator sponges accumulate moisture, dead skin, oils, eye shadows, and other substances and may provide a suitable reservoir for microorganisms which may be transferred to subsequent users. In one store, unused cotton-tipped swabs were available for customer use, but the swabs were not displayed and had to be requested. Single-use swabs are obviously the easiest and best way to eliminate two of the major contamination problems of tester trays. In addition, the use of tester trays with covers, prohibiting the use of fingers, and expiration dates of 1 year from the first display date should also contribute to improved hygiene and minimize microbial contamination of these trays.

Store personnel attested to the age of the testers sampled in this study. The in-use ages ranged from 6 to 24 months. Older testers were obviously exposed to more challenges by customers, and deterioration or inactivation of the inhibitors in the cosmetics may have occurred in some testers. A few testers were resampled days or weeks after the initial sampling. Sometimes similar levels of contamination with normal flora were detected when testers were re-

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sampled, and on other occasions no contamination was found. Failure to recover bacteria in previously positive tester wells may have been due to one or more of the following: lethal effects of drying or preservative action or both, subsequent customer sampling that removed contaminants, and sampling variations. Although not ideal, the swab rotation method of surface sampling and subsequent plating should give a realistic and representative semiquantitative approximation of microbial populations. Somewhat higher microbial counts might be expected if the entire tester were sampled instead of the 2.0- to 4.5-mg surface sample acquired by each swab. A comparative study was not possible for the following two reasons: the display shadows were expensive, and often no replacements were held in stock. Furthermore, no store manager expressed an interest in selling any of his used testers. Nevertheless, we feel that surface sampling probably gives a representative picture of the kinds and numbers of microbes found in these in-use testers.

In our in-use sampling survey, we found that brand A harbored both *S. aureus* and gram-negative rods, whereas only low levels of normal skin flora were recovered from brand B. When *S. aureus* was studied in challenge tests, it was unable to survive in either brand. The death of *S. aureus* probably was due to the action of preservatives, to a lack of nutrients, to drying conditions, or to a combination of two or three of these conditions.

An analysis of the data after the challenge of brand A with *A. calcoaceticus* clearly indicated growth in one of the two lots tested. This suggested that there was lot-to-lot variation in brand A.

P. aeruginosa was able to survive and multiply in both brands of eye shadow. Even when low levels of microorganisms were introduced, the powders had no obvious inhibitory effect. This behavior is suggestive of the generally resistant character of *P. aeruginosa* to antimicrobial agents and preservatives. The failure to recover *P. aeruginosa* from retail samplers may have been related to no or very rare introduction of these bacteria into the samplers. Variations in conditions between the experimental laboratory procedure and the actual in-use testers might also account for some of the differences.

Our results with *Acinetobacter* and *Pseudomonas* somewhat parallel the behavior of pseudomonads and *Acinetobacter* in aquatic environments (6, 8, 10, 14, 15, 16, 18). Eye shadows are obviously not aquatic environments, and inhibitors of microbial growth are generally present as additives (5, 22, 29). However, the presence of talcum and other hygroscopic agents in eye shadows attracts and binds water vapor to the cosmetics (7, 23, 24, 26). Furthermore, resistance of gram-negative bacteria to some microbial inhibitors has been reported previously (13). In the challenge studies, there was no apparent susceptibility of *P. aeruginosa* to any of the inhibitors that were present in the eye shadows.

When used alone as a control powder without any dyes or inhibitors, talcum permitted good growth of *P. aeruginosa* and *A. calcoaceticus*. These results parallel those obtained with *Escherichia coli* by Bigger and Nelson (7). These workers found that talcum and other inorganic compounds may adsorb gases such as CO_2 and NH_3 from the air. These gases are then available as nutrients for the growth of bacteria. It should be reemphasized that talcum is a basic ingredient of pressed-powder eye shadows (23, 24).

Eye shadows represent interesting matrices for the study of microbial interactions with artificially compounded substrates. Our data show that used display eye shadow testers in retail stores are often contaminated with microorganisms and that under laboratory conditions eye shadows seeded with certain gram-negative bacteria may occasionally permit microbial growth. The experimental challenge method described in this paper for the study of the growth or inhibition of microorganisms in eye shadows may be useful for the study of similar matrices.

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