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Mascara contamination: in use and laboratory studies

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Synopsis

Eye area cosmetics are subject to varied levels of microbial CONTAMINATION during their normal use. Selected MASCARAS with known preservative formulations were compared for their resistance to microbial colonization DURING USE by study groups and following a laboratory challenge test. A few products supported active growth of microorganisms after less than two weeks of normal use and after LABORATORY challenge. In study groups, the establishment of reproducing populations of microorganisms in certain mascaras occurred consistently with select individuals. Mascaras containing only parabens or imidazolidinylurea appeared to be less effective in retarding microbial growth than those containing formalin donors or mercurials. Effectiveness of preservative formulations may gradually decrease with the age of the product.

INTRODUCTION

Most mascaras contain antimicrobials to maintain the integrity of the product and to protect the consumer from the development of potentially harmful contaminants. Nevertheless, certain mascaras examined during use have been found to be overgrown with microorganisms and in some instances to be associated with eye infections (1–3). Ramp and Witkowski (4) have reviewed typical procedures for testing preservative systems in cosmetics. These involve the introduction of microorganisms, usually about 1×10^6 cells, into a 1-ml or 1-g sample of product. Effectiveness is determined by the recovery of less than 0.1 per cent of the inoculum after seven days. Sampling intervals may be extended for several months. Bruch (5) expressed concern about the lack of comparative data published on the various testing procedures. Yanagi (6) recommended that the challenge test for cosmetics be carried out in the commercial containers in which they are sold to the consumer. Laboratory evaluation of the effectiveness of the preservatives in mascaras by the usual microbiological procedures is difficult as many formulations are not readily solubilized by water. Dilution procedures used for enumerating microorganisms in mascaras may require the use of solvents or emulsifiers which may be toxic to the challenge microorganisms (7,8). At present, there is no standard procedure for evaluating the resistance of mascara formulations to microbial degradation. This report compares "in use" study-group analysis with labora-

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tory challenge tests to determine the effectiveness of preservative formulations in mascaras.

MATERIALS AND METHODS

MASCARAS

Mascaras were purchased at retail or secured directly from the manufacturer between 1975 and 1977. They were semi-solid, oily products of poor miscibility with water. The preservatives included in the mascaras are presented in Table 1. Concentrations of the microbial inhibitors were provided by the manufacturer and varied for the different brands. The total parabens per mascara ranged from 0.15 per cent to 0.5 per cent, imidazolidinylurea from 0.3 to 0.4 per cent and Dowicil 200 from 0.15 to 0.2 per cent. None of the mascaras contained over 60 ppm of mercury. Several mascaras were stored at room temperature for up to one year and at 37 and 56°C for seven days prior to laboratory challenge.

CHALLENGE ORGANISMS

Mascaras were challenged separately and sequentially with *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* initially isolated from used mascaras. The bacteria were grow at 37°C in tryptic soy broth (Difco) for 18 to 24 hr, harvested by centrifugation and washed three times in phosphate-buffered saline (PBS; NaCl 8.0 g, KCl 0.20 g, KH_2PO_4 0.12 g, Na_2HPO_4 0.91 g, deionized water 1 l, pH 7.2). The final pellet was resuspended in 10 ml of PBS, vortexed for 15 sec, sonicated at maximum power for 15 sec (Ratheon 5120A) and vortexed again for 15 sec. The bacterial suspension was diluted to an optical density (OD) between 0.4 and 0.7 at 600 nm for *S. epidermidis* and at 500 nm for *P. aeruginosa.* Appropriate dilutions of these solutions were made from a standard OD-colony-forming-unit curve to give approximately 10^6 colony-forming units (cfu) per ml. Viable counts were determined by spreading 0.1 ml of appropriate serial dilutions in triplicate on tryptic soy agar using PBS as a diluent. One ml containing 10^6 cfu was diluted to 6 ml with PBS. The 6-ml cell suspension was drawn into a syringe fitted with a Swinny filter holder (Millipore Corporation) and slowly forced through a 13-mm diameter membrane with an average pore size of 0.22 μm.

Table I
Preservative Content of Mascaras

	Mascara Code							
Preservatives	A	B	C	D	E	F	G	H
Methyl paraben	−	+	+	−	−	+	+	+
Propyl paraben	+	+	+	+	−	−	−	−
Butyl paraben	−	−	−	−	−	+	−	−
Dehydroacetic acid	−	+	−	−	+	−	+	−
Imidazolidinylurea	+	+	+	−	−	−	−	−
Dowicil 200	−	−	−	−	+	+	+	−
Phenylmecuric acetate	+	−	+	−	−	−	+	−

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MEMBRANE CHALLENGE

Mascara was removed with aseptic technique from its container and 300 to 400 mg was packed into a sterile glass cylinder 3 mm deep with an inner diameter of 13 mm. Two membranes were placed on the mascara. The membrane in direct contact with the mascara had an average porosity of 8 μm. This membrane served to retain the mascara in the well and to facilitate successive challenges with various microorganisms. The second membrane (0.22 μm porosity), containing the challenge organism (side with organism facing upwards), was placed over the first membrane. Periodically, the upper membrane was removed, replaced in the Swinny and back-flushed with 6 ml of buffered saline. The membrane was removed from the Swinny, placed in the 6 ml of saline, agitated and treated with mild sonication. The saline suspension was serially diluted and plated on appropriate media for enumeration of microorganisms. The same cylinder of mascara was challenged sequentially with membranes containing different organisms. Details of this procedure have been described elsewhere (3).

CONTAINER CHALLENGE

Suspension of microorganisms (1×10^6 cfu/g) was introduced directly into the original cosmetic container. The mascara was cultured periodically.

STUDY GROUPS

The study groups, each utilizing 15 to 25 college students, were conducted as previously described (2). Briefly, a single study group used a mascara of an identical lot of formulation for 9 to 11 weeks. The mascaras were cultured weekly for aerobic microorganisms and the history (number of times used, time elapsed since last use, individual habits, etc.) of use was recorded. If a mascara yielded over 50 cfu of the same contaminant on three consecutive samplings, it was withdrawn and a new mascara was issued. An incidence of contamination (IC) was recorded for each product. A single IC was defined as four or more colony-forming units of microorganisms per 3 to 8 mg of mascara present at least 4 hr after the last use. The IC was calculated from the total samplings of all mascaras used in the study groups.

Table II
Contamination of Mascaras During 9 to 11 Weeks of Use

Mascara	Incidence of Contamination	Number Established Contaminants/ Number Mascaras*
B	61**	6/13
C	6	0/17
D	33	0/22
F	7	0/17
G	9	0/18
H	23	0/21

*Established contaminants defined as presence of microorganisms at concentrations of at least 10^3 cells/mg ten days after last use.
**Percentage of samples yielding 4 cfu per 3 to 8 mg of mascara, at least four hours after last use.

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RESULTS AND DISCUSSION

An analysis of study-group results with selected mascaras is presented in Table II. Eight containers of mascara B yielded high numbers of *S. epidermidis* after an average of 22 uses. In excess of 10^5 cells/g was isolated from six of the mascaras ten days after their last use. In addition to *S. epidermidis,* three of these six each contained high numbers of either *Klebsiella pneumoniae, Pseudomonas putida* or *Candida parapsilosis.* These three mascaras were used by participants wearing hard contact lenses. These same participants, in study groups with other mascaras, usually demonstrated an IC above 20. The two mascaras (D, H) containing only parabens frequently yielded bacteria 4 to 8 hr after use, but no bacteria were recovered from these containers after they were stored for ten days.

The membrane challenge tests are summarized in Table III. As with the study groups, mascaras B, D, and H appeared to have relatively ineffective preservative systems. Mascaras B and D supported growth of the challenge organisms, whereas extended survival of the inoculum was found on mascara H. Direct challenge of organisms into the containers of these three mascaras with culture after seven days gave positive results only for mascara D with *P. aeruginosa.*

Two new lots of mascara B, one obtained just after production and one fortified with Dowicil 200 instead of imidazolidinylurea, and one new lot of mascara D supplemented with thimersol were obtained. These mascaras were given to small study groups of six to eight individuals and challenged with the double membrane procedure. The study group IC for the three was less than seven after 25 uses with none showing established contaminants. No organisms were recovered at three days with the membrane challenge test. Containers of these mascaras were stored (closed) for seven days at 37 and 56°C and then challenged with the membrane test. Only mascara B containing parabens and imidazolidinylurea (Table I) that had been kept at 37 and 56°C supported growth of bacteria. The other mascaras showed a slight decrease in inhibitory activity after heat treatment with 2 to 3 per cent of the challenge inocula recoverable after one day. A loss of 1 to 3 per cent in inhibitory activity was observed for mascaras A and F after one year of storage at room temperature. The exact age of mascara B tested in Table III is unknown. Mecurials and the formaldehyde preservative appeared more stable to heat than imidazolidinylurea.

Table III
Recovery of Challenge Organisms with Membrane Test

Challenge Organisms (1×10^6)	Mascaras								
	A	B	C	D	E	F	G	H	WP*
S. epidermidis	<0.1**	51	0	6	0	0	<0.3	9	37
P. aeruginosa	0	3	0	>100	0	0	0	2	14
*P. aeruginosa****	—****	>100	0	>100	0	0	0	46	15

*WP, white petrolatum control.

**Percentage of inoculum recovered after three days, mean of ten tests.

$$S^2 = \Sigma \frac{(x_i - \bar{x})^2}{n - 1}.$$

***Sequential challenge, initial challenge with *S. epidermidis.*

****—not done.

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Ramp and Witkowski (4) defined a well preserved cosmetic as one which would reduce a microbial challenge by at least 99.99 per cent in a relatively short period of time. If introduction of inocula directly into mascara containers is used to determine preservative effectiveness, the 99.99 per cent guideline may be obtainable as was shown in this study. This test, however, may not reflect the stability of the preservative system under conditions of use. In this preliminary analysis, the membrane test (Table III) appeared to correlate more closely with the in-use, study-group results (Table II) than with the container challenge test.

The membrane test was designed specifically for anhydrous products, but it appeared also to function with emulsion mascaras. The test permitted the sequential challenge of an aliquot of mascara with different microorganisms. Previous studies have suggested that contamination with *S. epidermidis* may make mascaras more susceptible to subsequent attack by pseudomonads (3). In this study, *P. aeruginosa* grew on mascara B following but not prior to challenge with *S. epidermidis*. Mascaras are applied frequently in humid bathrooms and condensation may occur in the mascara container. Cidal activity in this moisture layer appears necessary for the proper preservation of emulsion and anhydrous mascaras. Rapid death of the challenge organisms on the membrane may reflect the ability of the microbial inhibitors to diffuse and function in the microlayer of water on the surface of the cosmetic.

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