Microbial Contamination of Antibiotic Ophthalmic Ointments

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Abstract □ A survey was conducted to determine the sterility of antibiotic ophthalmic ointments. One hundred fourteen batches of ointments from 16 manufacturers were tested by the membrane-filtration procedure as described in the *Antibiotic Regulations*. Eight batches (7%) were found contaminated.

Keyphrases □ Ophthalmic antibiotic ointments—sterility testing □ Microbial contamination—ophthalmic antibiotic testing □ Membrane filtration—sterility testing

The *Antibiotic Regulations* (1) required a microorganism count for antibiotic ophthalmic ointments from 1947 to 1950. During that time, the incidence of contamination was very low as gauged by the following method: approximately 0.1 to 0.5 g. of ointment was squeezed onto the surface of 20 ml. of nutrient agar in a Petri dish. After the ointment was spread evenly over the agar with a sterile glass rod, the Petri dish was incubated at 37° for 48 hr. From the number of colonies appearing on the plate, the number of viable microorganisms per gram of ointment was calculated. The low incidence of contamination found was undoubtedly due to the poor sensitivity of this method. The test was deleted from the *Antibiotic Regulations* in 1950 (2).

In 1958, Vander Wyk and Granston (3) reported that of 28 samples of antibiotic ophthalmic ointments tested, 17 were nonsterile. Their finding of contamination in approximately 50% of the batches tested prompted a survey by Bowman and Holdowsky (4) who used the method described by Vander Wyk and Granston. This method included a dispersion step in which the ointment was shaken for 1 hr. at room temperature with sterile glass beads in 25 ml. of distilled water. Three 1.0-ml. aliquots were then placed in Petri dishes, mixed with melted blood agar, and incubated at 37° for 24 hr.

Forty-six samples representing 19 batches of ophthalmic ointments were tested in the survey and only two batches (10%) were found to be contaminated. Since the two contaminated batches were from the same manufacturer, it was concluded that the status of antibiotic ophthalmic ointments in general with respect to the degree and incidence of contamination appeared to be more satisfactory than the earlier report might have indicated. Although this method was an improvement over the earlier one (1), its weaknesses are twofold when it is used to test antibiotic ophthalmic ointments. First, some organisms may become encased in oil or fat and are thus denied access to nutrients required for survival and multiplication. Second, any organisms that may be released from the antibiotic ointment onto the culture medium encounter the antimicrobial activity of the antibiotic which might prevent their growth.

In an effort to develop an improved method for detecting and quantitating microorganisms in petrolatum-based ointments, Sokolski and Chidester (5) used a filtration procedure. Using aseptic technique, the ointments were first dissolved in isopropyl myristate and then filtered through bacterial-retentive membrane filters. The membranes were washed with special solutions to rid them from as much antibiotic activity as possible, and then they were transferred to absorbant pads in Petri dishes containing nutrient medium. Higher recoveries of microorganisms from seeded ointments were obtained by the filtration procedure than by methods previously used (1, 3). Sokolski and Chidester found that isopropyl myristate did not influence the viability of microorganisms (5); this finding was confirmed in this laboratory.

In 1964, the *Antibiotic Regulations* (6) were amended to include filtration procedures for the sterility testing of parenteral antibiotics. Subsequently, a filtration test for antibiotic ointments was developed which used isopropyl myristate as the diluting fluid. The *Antibiotic Regulations* (7) were again amended in 1965 to include a sterility test for ophthalmic ointments containing bacitracin, neomycin, and polymyxin when these ointments were labeled as sterile.

With the advent of this improved procedure for recovering microorganisms from petrolatum-based ointments, it seemed desirable to reinvestigate the incidence of microbial contamination in antibiotic ophthalmic ointments. Therefore, another survey was conducted. A limited number of commercially available ointments in which the antibiotics are incorporated in a plastibase (a hydrocarbon ointment base)[1] in lieu of petrolatum were not included in this survey, since they could not be tested by the filtration procedure.

EXPERIMENTAL

One hundred fourteen batches of antibiotic ophthalmic ointments from 16 different manufacturers were tested by the membrane-filtration procedure. All tubes were labeled to contain 0.125 oz. of ointment. Portions of 0.1 g. from each of 10 immediate ointment containers were aseptically transferred to 100 ml. of sterile isopropyl myristate at 47°. When 10 containers were not available, a 1.0-g. sample comprised of several portions from the tubes available was used. After the ointment was dissolved, the solutions were filtered as described for the membrane filtration sterility test (6, 7). Since it is difficult to wash residual antibiotics, especially neomycin, from membranes through which oleaginous preparations have been filtered, a rinse medium was devised to overcome this problem. The rinse medium contains bacteriological peptone and beef extract to protect the vegetative cells during the rinsing period. (Each filter membrane was rinsed five times with 100 ml. of the rinse medium.) Polysorbate 80 was included in the rinse medium to de-

[1] Jelene 50 W. This is an ointment base supplied by Research Products Corporation, Madison, Wisconsin.

Table I—Sterility Tests on Antibiotic Ophthalmic Ointments

Antibiotic in Ointment	Quant.	No. of Batches Negative	No. of Batches Positive	Organisms Recovered from Contaminated Batches
Bacitracin	500 units/g.	1	0	
Bacitracin Neomycin sulfate	500 units Equivalent to 3.5 mg. neomycin base/g.	2	0	
Bacitracin Neomycin sulfate	400 units Equivalent to 3.5 mg. neomycin base			
Polymyxin B sulfate	5,000 units/g.	24	5[a]	Batch[a] 1 Gram-positive bacilli 2 Gram-positive bacilli and mold[b] 3 Gram-positive bacilli 4 Gram-positive bacilli 5 *Penicillium sp.*
Bacitracin Neomycin sulfate	500 units Equivalent to 3.5 mg. neomycin base			
Polymyxin B sulfate Bacitracin Neomycin sulfate	5,000 units/g. 500 units Equivalent to 3.5 mg. neomycin base	4	0	
Polymyxin B sulfate	10,000 units/g.	3	1	*Mucor sp.*
Chlortetracycline HCl	10 mg./g.	6	0	
Erythromycin	5 mg./g.	5	0	
Neomycin sulfate	Equivalent to 3.5 mg. neomycin base/g.	36	0	
Oxytetracycline HCl Polymyxin B sulfate Neomycin sulfate	5 mg./g. 10,000 units/g. Equivalent to 3.5 mg. neomycin base	12	0	
Polymyxin B sulfate	6,000 units/g.	3	1	*Aspergillus sp.*
Penicillin G	1,000 units/g.	5	1	*Penicillium sp.*
Penicillin G	100,000 units/g.	1	0	
Tetracycline HCl	10 mg./g.	4	0	

[a] Batch Nos. 1–4 were from the same manufacturer. [b] Molds were not identified.

crease the filtration time. For ointments containing neomycin, sodium chloride, which is known to desorb neomycin from cellulose (8), was added to the rinse medium. As an additional precaution, two known inhibitors of neomycin activity, sodium chloride (8) and ascorbic acid (9), were added to the two growth media used, fluid thioglycollate and Sabouraud's liquid broth. For ointments without neomycin, the sodium chloride and ascorbic acid were omitted from the growth media. If the ointment contained penicillin, penicillinase (1,000 Levy units) was added to the 500 ml. of rinse medium and to each tube of growth media.

RESULTS AND CONCLUSIONS

As shown in Table I, contamination was detected in eight of the 114 batches tested. Of the eight contaminated batches, four were contaminated with molds, three with Gram-positive bacilli, and one with both Gram-positive bacilli and molds. To assure that these findings were not caused by laboratory manipulations, repeat tests were performed on each contaminated batch and in each case the identical organism was recovered on the second test. Although the incidence of contamination in this survey is only 7% compared to 10% in the earlier survey conducted by Bowman and Holdowsky, the finding of any contamination in ophthalmic ointments is considered extremely undesirable (10).

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