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Microbial Contamination of In-use Ophthalmic Preparations and Its Prevention

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Abstract **Aims:** To clarify the usefulness of preservative-free ophthalmic preparations equipped with a filter. **Methods:** A total of 1,615 samples of in-use ophthalmic preparations were examined for microbial contamination. **Results:** Of 1,094 samples of preservative-containing ophthalmic preparations, 31 (2.8%) showed microbial contamination. Of 289 samples of preservative-free ophthalmic preparations without a filter, 6 (2.1%) were contaminated, consisting of 4 (13.8%) of 29 samples of hospital preparations and 2 (0.8%) of 260 samples of commercially available new quinolone antimicrobial agents. On the other hand, the microbial contamination rate in preservative-free ophthalmic preparations equipped with a filter was 0% (0 of 232 samples).The major contaminants detected in these preservative-containing ophthalmic preparations and preservative-free ophthalmic preparations without a filter were glucose-nonfermentative Gram-negative bacilli such as *Pseudomonas fluorescens*, *Acinetobacter* spp., and *P. aeruginosa*, coagulase (−) staphylococci, and *Candida* spp. The contaminant level was 10-99 colony forming units (CFU)/mL in 37.8% (14 of 37 samples), and 10^2-10^6 CFU/mL in 62.2% (23 of 37 samples). **Conclusions:** Preservative-free ophthalmic preparations equipped with a filter not only have zero risk of the oculotoxic effects of preservatives, but are also safe in terms of their lack of microbial contamination.

Key words: ophthalmic preparation, multiple-dose, preservative, filter, microbial contamination

Introduction

Multiple-dose ophthalmic preparations that are used many times are classified into preservative-containing ophthalmic preparations (Type A) and preservative-free ophthalmic preparations without a filter (Type B) and preservative-free ophthalmic preparations equipped with a filter (Type C). There have been many reports on the microbial contamination of Type A or B, and the risk of ocular infection due to contaminated ophthalmic preparations has also been reported.[1-7] On the other hand, Type C are equipped with a membrane filter for the purpose of preventing microbial contamination during use. As a major advantage of this form of preparation, they do not require preservatives, and can also be used by patients with hypersensitivity or allergy to preservatives. However, there have been few studies on the microbial contamination of Type C.[8] Therefore, there is little evidence supporting that this preparation form is free from infection. The risk of ocular infection due to Type C is unclear.

To clarify the usefulness of Type C, we evaluated the microbial contamination rate, contaminant level, and species in various

types of ophthalmic preparation including Type C.

Methods

Investigated Ophthalmic Preparations and Their Collection Methods

We collected ophthalmic preparations that were personally used by outpatients and inpatients at the ophthalmological department of Yamaguchi University hospital (736 beds) between April 1, 2013 and March 31, 2014. The period from the first administration to the day the ophthalmic preparations were examined was to 1-6 months. A total of 1,615 samples of multiple-dose ophthalmic preparations (product volume, 2.5-10 mL) were examined, including 1,094 samples of commercially available Type A, 289 samples of Type B (hospital preparations, commercially available new quinolone antimicrobial agents) and 232 samples of commercially available Type C (Table 1). Hospital preparations were defined as ophthalmic preparations that are not commercially available, and were aseptically prepared using drugs for injection or reagents in the hospital. In addition, it was indicated that hospital preparations should be refrigerated during use by our pharmaceutical service. Type C are ophthalmic preparations in a container equipped with a membrane filter (0.22 μm) for the filtration of solutions when they are used.[9]

Concerning the collection of ophthalmic preparations, outpatients and inpatients were given a written explanation that the purpose of the collection of ophthalmic preparations is the "investigation of the state of in-use ophthalmic preparations," and their presentation of these preparations was voluntary. We consulted the ethics review committee, and got the reply of "review unnecessity" because of non-use of the patient's medical record and biological sample in this study.

Identification and Quantification of Contaminants

When 1 mL or more of ophthalmic solution was considered to remain in the container, the container was manually shaken for one minute, and solution obtained by the routine ophthalmic solution dropping procedure was used as the test solution. When the volume of the remaining ophthalmic solution was

Table 1 Therapeutic categories of evaluated ophthalmic preparations (n=1615)

Type	Pharmacology	Ophthalmic preparations	Number of examined samples	Total number of examined samples
Preservative-containing ophthalmic preparations	anti-glaucoma agents	brimonidine, latanoprost etc.	282	1094
	agents used for tests	tropicamide + phenylephrine	192	
	corticosteroid agents	betamethasone, fluorometholone	178	
	non-steroidal anti-inflammatory agents	diclofenac, bromfenac	133	
	antimicrobial agents	cefmenoxime	114	
	agents for corneal epithelial damage	sodium hyaluronate, diquafosol etc.	91	
	agents with other effects	pirenoxine, cyanocobalamin etc.	104	
Preservative-free ophthalmic preparations (hospital preparation [a])	antimicrobial agents	fluconazole, vancomycin etc.	18	289
	immunosuppressant agents	ciclosporin	9	
	agents with other effects	saline	2	
Preservative-free ophthalmic preparations (commercially available new quinolone antimicrobials)	antimicrobial agents	levofloxacin, gatifloxacin	260	
Ophthalmic preparations equipped with a filter [b]	corticosteroid agents	betamethasone	195	232
	anti-glaucoma agents	carteolol, timolol	37	

[a] Ophthalmic preparations aseptically prepared using drugs for injection and reagents in the hospital.
[b] Ophthalmic preparations allowing instillation of ophthalmic solution filtered through a 0.22 μm membrane filter that has been applied to the ophthalmic preparation container.

considered to be less than 1 mL, 1 mL of physiological saline was added to the ophthalmic solution in the clean bench using the following procedure. For Type C, the bottom of the container was disinfected with 80 vol% ethanol, and saline was injected using a syringe for injection. For the other types of ophthalmic preparation, saline was injected from the nozzle of the container using a syringe for injection. The containers were manually shaken for one minute, and solution obtained by dropping was used as the test solution. Each of the samples was diluted 10-fold and 100-fold in sterile saline. Subsequently, 0.2 mL of each dilution and of an undiluted sample were plated onto Trypto-Soy agar, SCDLP agar (each agar, Nippon Becton Dickinson Co., Tokyo, Japan), and Sabouraud Dextrose agar (Eiken Chemical Co., Tokyo, Japan). Plates were incubated at 30 ℃ for 24-72 hours (Trypto-Soy agar and SCDLP agar), or for 2-7 days (Sabouraud Dextrose agar). Bacterial species were identified using Gram staining, the OF tests, catalase tests, and cytochrome oxidase tests, and Api20 NE, Api20CAUX, VITEK® 2 Compact (bioMerieux Co., France).

Statistical Analysis

The association between the types of multiple-dose ophthalmic preparation and microbial contamination rate was analyzed using the χ^2 test. $P < 0.05$ was regarded as significant.

Results

The microbial contamination rate in the evaluated ophthalmic preparations was 2.8% (31 of 1,094 samples) in Type A, 2.1% (6 of 289 samples) in Type B, and 0% (0 of 232 samples) in Type C. The microbial contamination rate in Type C was significantly lower than that in Type A or B ($p = 0.03114$). The microbial contamination rate in Type A according to the preservative was 0.9% (1 of 110 samples) for 0.5% chlorobutanol, 1.6% (4 of 258 samples) for 0.02-0.07% *p*-hydroxybenzoate esters, 3.1% (20 of 647 samples) for 0.001-0.02% benzalkonium chloride, and 7.6% (6 of 79 samples) for 0.005% sodium chlorite. Microbial contamination was observed in the ophthalmic preparations containing each type of preservative. The microbial contamination rate in Type B was 13.8% (4 of 29 samples) in the hospital preparations and 0.8% (2 of 260 samples) in the commercially available new quinolone antimicrobial agents; microbial contamination was observed in both hospital and commercially available preparations (Table 2). The highest microbial contamination rate in Type

Table 2 Microbial contamination of in-use ophthalmic preparations (n=1615)

Type	Preservative, concentration	Ophthalmic preparations	Number of samples showing microbial contamination[a] / number of evaluated samples (%)	Total number of samples showing microbial contamination/total number of evaluated samples (%)
Preservative-containing ophthalmic preparations	sodium chlorite, 0.005%	brimonidine	6 / 79 (7.6)	31 / 1094 (2.8)
	benzalkonium chloride, 0.001-0.02%	sodium hyaluronate, dorzolamide, fluorometholone, etc.	20 / 647 (3.1)	
	p-hydroxybenzoate esters, 0.02-0.07%	betamethasone, cefmenoxime, etc.	4 / 258 (1.6)	
	chlorobutanol, 0.5%	diclofenac	1 / 110 (0.9)	
Preservative-free ophthalmic preparations	free	hospital preparations; fluconazole, amphotericin B, saline, etc.	4 / 29 (13.8)	6 / 289 (2.1)
		commercially available new quinolone antimicrobials; levofloxacin, gatifloxacin	2 / 260 (0.8)	
Ophthalmic preparations equipped with a filter	free	betamethasone, carteolol, timolol	0 / 232 (0)	0 / 232 (0)

[a] 5- colony forming units (CFU)/mL were defined as microbial contamination.

A according to the pharmacology was 9.9% (8 of 91 samples) for agents to treat corneal epithelial damage. The microbial contamination rate for agents to treat corneal epithelial damage was significantly higher than that of other agents ($p = 0.00076$).

The contaminants and their levels in the contaminated Type A or B (total, 37 samples) are shown in Table 3. The detected contaminants were Gram-negative bacilli in 18 (48.6%) of the 37 samples. The major bacterial species were *Pseudomonas fluorescens, Acinetobacter* spp., *Rahnella aquatilis*, and *P. aeruginosa*. Two (5.4%) of the 37 samples were contaminated by *P. aeruginosa*, and both were ophthalmic preparations containing benzalkonium chloride. Gram-positive cocci were identified in 12 (32.4%) of the 37 samples, most of which were coagulase (−) staphylococci. In addition, fungi were observed in 11 (29.7%) of the 37 samples, and the major contaminants were *Candida* spp. and filamentous fungi. The contaminant level was 10-99 colony forming units (CFU)/mL in 37.8% (14 of the 37 samples) and 10^2-10^6 CFU/mL in 62.2% (23 of the 37 samples). The contamination at a 10^2-10^6 level was observed in 13.8% (4 of 29 samples) of hospital preparations (Type B), 5.1% (4 of 79 samples) of the ophthalmic preparations containing sodium chlorite (Type A), 1.9% (12 of 647 samples) of those containing benzalkonium chloride (Type A), 0.8% (2 of 258 samples) of those containing *p*-hydroxybenzoate esters (Type A), and 0.4% (1 of 260 samples) of commercially available new quinolone antimicrobial agents (Type B). Of Type A, only those containing chlorobutanol did not show contamination at the 10^2-10^6 CFU/mL level.

Discussion

Multiple-dose ophthalmic preparations are classified into 3 types: preservative-containing ophthalmic preparations (Type A), preservative-free ophthalmic preparations without a filter (Type B) and preservative-free ophthalmic preparations equipped with a filter (Type C). Of 1,094 samples of Type A, 31 (2.8%) showed microbial contamination. Microbial contamination was observed in preparations containing each preservative. Other studies have shown that the presence of preservatives in ophthalmic preparations is inadequate for the prevention of microbial contamination of these preparations.[1-3] This survey also suggested that microbial contamination cannot be prevented in Type A. The microbial contamination rate for the agent to treat corneal epithelial damage was highest out of all examined Type A. Therefore, it is concerned that the use of microbial contaminated agents may cause eye infection. *P. aeruginosa* is an important contaminant in ophthalmic preparations, inducing corneal ulcers.[10-13] The contamination of *P. aeruginosa* was observed even in Type A. In addition, the rate of microbial contamination at a 10^2 CFU/mL level or higher in contaminated samples according to the preservative was 66.7% (4 of 6 contaminated samples) for sodium chlorite, 60% (12 of 20) for benzalkonium chloride, and 50% (2 of 4) for *p*-hydroxybenzoate esters. When the contamination level in ophthalmic preparations is less than 10^2 CFU/mL, the contamination is considered to be due to contact between the tip of the ophthalmic preparation container and the finger or eyelid.[14] However, contamination at this level or higher suggests microbial growth in ophthalmic preparations. In this study, the contaminants detected in the ophthalmic preparations showing microbial contamination at a 10^2 level or higher were often Gram-negative bacilli. Gram-positive cocci do not grow well with a small amount of nutrients that are present in intravenous fluids, but Gram-negative bacilli do grow.[15,16] This may be the reason for the growth of Gram- negative bacilli in ophthalmic solutions. Thus, it is possible that Type A can be contaminated by microorganisms including highly toxic ones to the eye such as *P. aeruginosa*, and the microorganisms grow in the preparations.

Of 289 samples of Type B (hospital preparations, commercially available new quinolone antimicrobial agents), 6 (2.1%) showed microbial contamination. In particular, 4 (13.8%) of 29 samples of hospital preparations were contaminated, and this contamination rate was the highest among all types of ophthalmic preparation. Hospital preparations, which do not contain preservatives, have been reported to be associated with a high risk of microbial contamination,[6,7] which was supported by this

Table 3 Species and level of contaminants detected in ophthalmic preparations showing microbial contamination

Sample number	Ophthalmic preparations	Preservative	Contaminants	Contaminant level (CFU/mL)
1	saline (hospital preparation)	free	*Serratia liquefaciens,* etc.	3.1×10^{6}
2	fluconazole (hospital preparation)	free	*Rahnella aquatilis*	9.9×10^{5}
3	saline (hospital preparation)	free	*Rahnella aquatilis*	8.5×10^{5}
4	latanoprost	benzalkonium chloride	*Pseudomonas fluorescens*	7.5×10^{5}
5	latanoprost	benzalkonium chloride	*Pseudomonas fluorescens*	2.8×10^{5}
6	sodium hyaluronate	benzalkonium chloride	*Chryseomonas indologenes*	7.2×10^{4}
7	sodium hyaluronate	benzalkonium chloride	*Chryseomonas indologenes*	4.2×10^{4}
8	cefmenoxime	*p*-hydroxybenzoate esters	*Stenotrophomonas maltophilia,* etc.	2.9×10^{4}
9	sodium Hyaluronate	benzalkonium chloride	*Candida parapsilosis*	1.1×10^{4}
10	carteolol	benzalkonium chloride	*Pseudomonas aeruginosa*	6.0×10^{3}
11	ketotifen	benzalkonium chloride	*Pseudomonas fluorescens,* etc.	2.9×10^{3}
12	sodium hyaluronate	benzalkonium chloride	*Enterobacter cloacae*	2.1×10^{3}
13	sodium hyaluronate	benzalkonium chloride	coagulase (−) staphylococci	1.2×10^{3}
14	latanoprost	benzalkonium chloride	*Bacillus* spp.	540
15	betamethasone	*p*-hydroxybenzoate esters	coagulase (−) staphylococci	470
16	pirenoxine	benzalkonium chloride	*Pseudomonas aeruginosa,* etc.	460
17	brimonidine	sodium chlorite	*Candida zeylanoides,* etc.	435
18	sodium hyaluronate	benzalkonium chloride	*Pantoea* spp.	410
19	brimonidine	sodium chlorite	*Candida zeylanoides*	220
20	amphotericin B (hospital preparation)	free	*Candida zeylanoides*	190
21	gatifloxacin (commercially available new quinolone antimicrobial)	free	*Cryptococcus albidus*	170
22	brimonidine	sodium chlorite	*Gardnerella vaginalis*	170
23	brimonidine	sodium chlorite	*Pseudomonas fluorescens,* etc.	125
24	sodium hyaluronate	benzalkonium chloride	*Micrococcus luteus / lylae*	90
25	pirenoxine	benzalkonium chloride	*Burkholderia cepacia* etc.	90
26	pirenoxine	benzalkonium chloride	coagulase (−) staphylococci	85
27	betamethasone	*p*-hydroxybenzoate esters	*Kocuria kristinae* etc.	80
28	brimonidine	sodium chlorite	filamentous fungi	70
29	sodium hyaluronate	benzalkonium chloride	*Pseudomonas fluorescens*	60
30	oxybuprocaine	benzalkonium chloride	coagulase (−) staphylococci	60
31	cefmenoxime	*p*-hydroxybenzoate esters	*Acinetobacter baumannii/ calcoaceticus*	35
32	bromfenac	benzalkonium chloride	coagulase (−) staphylococci	35
33	diclofenac	chlorobutanol	coagulase (−) staphylococci	25
34	brimonidine	sodium chlorite	filamentous fungi	15
35	levofloxacin(commercially available new quinolone antimicrobial)	free	*Candida albicans*	15
36	artificial tears	benzalkonium chloride	coagulase (−) staphylococci	10
37	tropicamide + phenylephrine	benzalkonium chloride	coagulase (−) staphylococci	10

study. Of the 4 contaminated hospital preparations, 2 showed contamination at the 10^5 CFU/mL level, and 1 showed contamination at the 10^6 CFU/mL level. These results suggest that hospital preparations have a high risk of microbial contamination, and microorganisms can grow in these preparations. Therefore, during use of Type B as hospital preparations, their strict cold storage is necessary. Of 260 samples of commercially available new quinolone antimicrobial agents, 2 (0.8%) showed microbial contamination. Although the contamination rate is low, microbial contamination could not be prevented even in ophthalmic preparations containing new quinolone antimicrobials.

On the other hand, none of the 232 samples of Type C showed microbial contamination. To our knowledge, there has been only one study on the microbial contamination of in-use Type C, and 20 samples were evaluated in this study.[8] In the present study, we evaluated microbial contamination in an increased number of samples of in-use Type C, and confirmed that this ophthalmic preparation form is appropriate for the prevention of microbial contamination. The membrane filter that has been applied to the inside of the container of ophthalmic preparations may contribute to the prevention of microbial contamination. Additional investigations about the contamination of *Mycoplasma* or *Chlamydia* should be performed.[17,18]

A major advantage of Type C is the absence of preservatives. Basic studies have shown that preservatives are toxic to corneal epithelial and endothelial cells and conjunctival epithelial cells.[19-21] A clinical study also showed that the incidence of corneal epithelial disorder in eyes early after corneal transplantation was lower in a group using Type C than in a group using Type A despite the same medicinal properties.[22] Another study suggested that preservatives in Type A are sometimes the cause of disorders on the eye surface, such as dry eye in patients with glaucoma.[23] In addition, benzalkonium chloride, frequently used as a preservative for ophthalmic preparations, induces hypersensitivity and allergic reactions (conjunctival congestion, tearing, or burning and stinging sensations). Indeed, patients who used ophthalmic preparations containing benzalkonium chloride and developed anaphylaxis symptoms (such as dyspnea and corneal abrasion) have been reported.[24,25] Other studies have shown that benzalkonium chloride contained in nasal drops or inhalation solutions caused anaphylaxis symptoms.[26,27] Based on these reports, preservative-free ophthalmic preparations are desirable.

There are single- and multiple-dose ophthalmic preparations. Single-dose preparations, which do not contain preservatives, are, as with preparations equipped with a filter, safe for the eyes. However, the disadvantage of these single-dose preparations is their high cost compared with Type C. Preparations equipped with a filter, which are multiple-dose preparations, are also excellent in terms of cost-effectiveness compared with single-dose ophthalmic preparations. However, concerning the disadvantages of Type C compared with other conventional preparations, the container is large, and the solution is slightly difficult to drop, and the manufacturing cost is high. In the future, after overcoming these disadvantages, Type C will further contribute to safe treatment.

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Conflict of Interest

The authors state no conflict of interest.

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