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Association of *Pseudomonas* and *Serratia* Corneal Ulcers with Use of Contaminated Solutions

M. S. MAYO,[1]† R. L. SCHLITZER,[2] M. A. WARD,[3] L. A. WILSON,[3] AND D. G. AHEARN[1]*

Laboratory for Microbial and Biochemical Sciences, Georgia State University, Atlanta, Georgia 30303[1]; Alcon Laboratories, Inc., Fort Worth, Texas 76134[2]; and Department of Ophthalmology, Emory University School of Medicine, Atlanta, Georgia 30322[3]

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The wetting and soaking solutions and contact lens cases of eye clinic patients commonly were contaminated with gram-negative bacteria during their use. *Serratia marcescens* occurred most frequently in preserved solutions, whereas *Pseudomonas aeruginosa* was most often recovered from home-prepared saline. The bacteria were recovered at densities of $>10^6$ cells per ml and typically persisted in the solutions. Eight patients who developed bacterial keratitis during 1986 used solutions contaminated with the etiological agents of the infections. Improper hygienic practices of the patients and failure of some preservative systems were implicated in the development of the infections.

Recent investigations have documented an increased incidence of *Pseudomonas aeruginosa* keratitis associated with hydrogel contact lens wear (2, 6). These infections have been related to contact lens damage to the corneal epithelium from anoxia or ill-fitted lenses, situations that predispose the eye to infection (3, 7). Improper hygienic practices and the use of contaminated solutions have been reported to be involved in the etiology of bacterial keratitis among contact lens wearers (10). Wilson et al. (12) and Mayo et al. (5) have demonstrated that the isolates of *P. aeruginosa* from corneal ulcers and contaminated saline solutions of a given patient were of the same strain. *Serratia marcescens*, which is currently the etiological agent in approximately 5 to 10% of gram-negative corneal ulcers, also has been found in contact lens solutions of keratitis patients (4, 7, 8). The persistence of *S. marcescens* in variously preserved contact lens solutions has been reported, but clinical implications of these findings have not been resolved (1, 9, 11). To explore further the possible link of an increased incidence of *Pseudomonas* and *Serratia* keratitis to the use of contaminated ocular solutions, we have examined various ocular solutions of 75 patients, including solutions of 8 patients (examined by L.A.W.) who were diagnosed with corneal ulcers during 1986.

MATERIALS AND METHODS

Various ophthalmic solutions from different manufacturers used by patients visiting the Emory Eye Clinic and unused solutions from commercial sources were screened for microbial growth. One drop each of the contact lens solutions and other ocular solutions was inoculated directly from the container onto chocolate agar, and the inoculum was swabbed over the surface of the agar. Selected products were also sampled aseptically through the wall of the container with a needle and syringe. These products were serially diluted in Dey Engley agar (Difco Laboratories, Detroit, Mich.) and were plated onto nutrient agar. Corneal ulcer scrapings were inoculated onto chocolate agar and Sabouraud dextrose agar and were immersed into thioglycolate broth. Plates were incubated at 37°C in a candle jar for 24 h, followed by aerobic incubation for 24 h. When possible, those solutions, in use by patients, that were found to be contaminated were collected kept on the laboratory shelf and were replated periodically (3- to 5-month intervals) for up to several years. Isolates were characterized by their Gram stain reaction and by biochemical reactions on the API 20E system (Analytab Products, Inc., Plainview, N.Y.), the Vitek Computerized System (Vitek System, Inc., Hazelwood, Mo.), and the following standard tests: urea hydrolysis, *O*-nitrophenyl-β-*O* production, carbohydrate assimilation for arabinose, maltose, and xylose, and hemolysis in blood. Antimicrobial susceptibilities were determined by the Vitek Computerized System. Antimicrobial agents tested were amakacin, ampicillin, carbenicillin, cefamandole, cefoxitin, cephalothin, chloramphenicol, gentamicin, tetracycline, tobramycin, and trimethoprim-sulfamethoxazole. Plasmid profiles were obtained as previously described by Mayo et al. (5).

Case histories of patients with bacterial keratitis. Patient A, a 32-year-old computer operator, developed an *S. marcescens* corneal ulcer in the grafted tissue of a corneal transplant. The ulcer was located at the site of a protruding corneal suture. After the removal of more sutures 2 months later, the patient resumed wearing his rigid, gas-permeable lens; contrary to his physician's advise, he continued use of old solutions. He developed a second *S. marcescens* corneal ulcer 2 days later. Patient B developed a *Serratia* corneal ulcer while wearing rigid, gas-permeable lenses. His wetting and soaking solution also was contaminated with *S. marcescens*; other details of his history were not available. Patient C, a 41-year-old civil engineer with a history of keratoconus, wore hard contact lenses for 22 years. He underwent a corneal transplant in his left eye. He continued wearing his old, hard contact lens in his right eye and was prescribed a rigid, gas-permeable lens for his left eye. He developed a *Serratia* corneal ulcer in his right eye. Patient D, a 72-year-old retiree, did not wear contact lenses. He developed a *Serratia* corneal ulcer 2 months after a corneal transplant. He was diagnosed with a second *Serratia* corneal ulcer 2 months after the first ulcer resolved.

Patients E to H wore cosmetic, hydrogel lenses. Patient E, a 39-year-old operating room technician, wore soft extended-wear contact lenses for 14 months prior to a *P. aerugin-*

* Corresponding author.
† Present address: Radiation Sterilizers, Inc., Decatur, GA 30035.

TABLE 1. Incidence of gram-negative bacteria in solutions used by patients

Solution type	Incidence (no. contaminated/ no. sampled)	Predominant microorganisms (no. of solutions contaminated)
Nonpreserved saline	9/11	*P. aeruginosa* (5), *E. agglomerans*, *S. marcescens*
Preserved wetting and soaking solutions	24/58	*S. marcescens* (11), *K. ozaenae* (2), *P. aeruginosa* (2), *E. cloacae* (3)
Lens cases with various solutions	19/44	Mixed gram negatives and gram positives
Steroid suspensions	2/8	*S. marcescens* (2)
Preserved saline	4/14	*P. aeruginosa*, *P. maltophila*
Artificial tears	3/32	*Pseudomonas* spp.
Cleaning solution	3/46	*S. marcescens*, *S. liquefaciens*, *E. aerogenes*
Disinfectant	0/9	
Miscellaneous[a]	0/16	

[a] Mainly ocular medicants.

osa ulcer. He used an irrigating saline solution (obtained from the operating room) for rinsing, heat disinfection, and eye drops. The patient irregularly transferred the irrigation solution to different containers. Patient F, a 24-year-old house painter, had worn soft lenses for 6 years (5 years of daily-wear lenses, followed by 1 year of extended-wear use) prior to developing a *Pseudomonas* corneal ulcer. His lens case was visually dirty. He used no disinfection when he removed his lenses every 2 weeks. He cleaned his lenses with enzymes and then stored them in sorbate-preserved saline solution until reinsertion. Patient G, a 29-year-old medical practitioner, had worn soft daily-wear contact lenses (38% water content) for 15 years prior to developing a *Pseudomonas* corneal ulcer. His lens case was visually dirty, and his gallon jug of distilled water for preparation of saline (half used) yielded over 10^6 bacteria per ml. He used no disinfection, and he stored his lenses in sorbate-preserved saline. Patient H, a 23-year-old medical student, wore soft extended-wear contact lenses (55% water content) on an intermittent basis for 6 months before he developed a *Pseudomonas* corneal ulcer. He used a hydrogen peroxide system for disinfection. Although he used a preserved saline as artificial tears, he transferred the saline from the original container to a smaller, nonsterile applicator bottle. Patients E to H used their saline solutions as artificial tears. All patients received topical fortified gentamicin therapy, and the infections were resolved.

RESULTS

In-use ocular solutions from 75 patients commonly yielded gram-negative bacteria (Table 1). Between 40 and 80% of the soaking and wetting solutions, contact lens cases, and nonpreserved salines contained microorganisms. The lens cases contained higher densities and larger varieties of bacteria. *S. marcescens* was the most common organism in preserved solutions, occurring in 11 of the 24 contaminated soaking and wetting solutions and in the 2 contaminated ophthalmic steroid suspensions. *Serratia* frequently was the only species isolated from the preserved solutions. Other common microorganisms isolated included *Enterobacter cloacae*, *P. maltophilia*, and *Klebsiella ozaenae*. *Pseudomonas* spp., particularly *P. aeruginosa*, occurred in 55% of the nonpreserved salines. It was also commonly encountered in the lens cases of the same patients. Most often, cell densities in the contaminated solutions, as determined by dilution procedures, exceeded 10^6 cells per ml.

Of eight patients with bacterial keratitis, all were found to be using contaminated solutions (Table 2). *S. marcescens* was the etiological agent and the contaminant in the preserved solutions, whereas *Pseudomonas* sp. was associated with saline solutions. This distribution in preserved and nonpreserved solutions was not observed with the examination of solutions from patients with conjunctivitis or without symptoms. *S. marcescens* was isolated from a chlorhexidine-preserved wetting and soaking solution and from home-prepared saline of two patients with conjunctivitis, whereas *P. aeruginosa* was obtained from the chlorhexidine-preserved solutions of two patients who exhibited no symptoms. *P. aeruginosa* was isolated from a swab of the conjunctiva of one of these latter two patients. As indicated previously, cell densities exceeded 10^6 cells per ml even when solutions or lens cases appeared clean.

New products representing the various categories tested, including 25 soaking and wetting solutions, also were screened for microorganisms. None of the new products yielded microorganisms. Random sampling of 151 solutions of 26 patients indicated that about 12% of these in-use solutions yielded gram-negative rods. Products followed while they were in use became contaminated with reproduc-

TABLE 2. Infectious corneal ulcers reviewed in 1986

Patient code[a]	Age (yr)	Type of lens[b]	Etiological agent	Contaminated solution		
				Type	Preservative[c]	Dominant organism
A	32	RGP	*S. marcescens*	Wetting solution	BC	*S. marcescens*
B	Unknown	RGP	*S. marcescens*	Wetting and soaking solution	CXE	*S. marcescens*
C	41	RGP	*S. marcescens*	Wetting and soaking solution	CXE	*S. marcescens*
D	72	None	*S. marcescens*	Steroid solution	BC	*S. marcescens*
E	39	SEW	*P. aeruginosa*	Lens case solution	None	*P. aeruginosa*
				Irrigating saline	None	*P. maltophilia*
F	24	SEW	*P. aeruginosa*	Lens case solution	None	*P. aeruginosa*
G	29	SDW	*P. aeruginosa*	Lens case solution	None	*P. aeruginosa*
				Preserved saline	S	*P. maltophilia*
				Distilled water	None	*P. cepacia*
H	23	SEW	*P. aeruginosa*	Home-prepared saline	None	Mixed gram negatives
				Lens case solution	None	*P. aeruginosa*

[a] All patients were male.
[b] RGP, Rigid, gas-permeable; SEW, soft, extended wear; SDW, soft, daily wear.
[c] BC, Benzalkonium chloride; CXE, chlorhexidine; S, sorbic acid.

ing populations, generally within 1 month of their distribution to the patient.

Representative preserved solutions that became contaminated were maintained in the laboratory and cultured after 1 to 6 months. The contamination persisted in all of the 18 solutions retested without evidence of marked reduction in cell densities. In a few instances, cell densities increased. Cell densities determined for fluids in containers with an elongated tip, such as the steroid suspension, were of a two- to fivefold greater magnitude when the suspension was expressed through the applicator tip as contrasted with densities obtained for suspensions withdrawn aseptically through the wall of the container. Eight contaminated hard contact lens wetting and soaking solutions that had been maintained in the laboratory for over 3 years from a previous study (1) also contained densities of *S. marcescens* of $>10^6$ cells per ml.

Isolates of *P. aeruginosa* from the corneal ulcer and the saline of the same patient were available for only two of the four keratitis cases. The paired isolates were of the same serotype (patient E, serotype O:11; patient F, serotype O:10). Both corneal ulcer and saline isolates of patient F lacked plasmids. The isolate from the corneal ulcer of patient E had two plasmids (8 and 12 megadaltons), but the isolate from the saline lacked plasmids. None of the eight representative isolates examined was resistant to gentamicin or tobramycin.

Most isolates of *S. marcescens* were nonpigmented. All 19 representative isolates examined during 1986 were susceptible to amikacin, gentamicin, tobramycin, and trimethoprim-sulfamethoxazole; all were resistant to cefamandole, cephalothin, and chloramphenicol. Some resistance to carbenicillin and cefoxitin was noted. These 1986 isolates were all nonpigmented, and none assimilated lactose, contrary to most isolates obtained during the previous 5 years. Except for one isolate from a sorbic acid-preserved wetting solution, none demonstrated the presence of plasmids.

DISCUSSION

The selective nature of our study group (all patients using contact lenses or developing infectious corneal ulcers during 1986) does not permit us to extrapolate the demographics of this study to a general population. Nevertheless, used ocular solutions, particularly of contact lens wearers were commonly contaminated with mixed populations of microorganisms. *S. marcescens* was the most common contaminant in preserved solutions, whereas pseudomonads were more common in nonpreserved saline solutions. The *Pseudomonas* and *Serratia* spp. present in the contaminated ocular solutions of our patients were probably not of nosocomial origin since they were susceptible to tobramycin and gentamicin. Both *Serratia* and *Pseudomonas* spp. appeared to be established as reproducing populations in ocular products after only a few uses and persisted in these solutions for prolonged periods. Two patients (patients A and D) demonstrated successional corneal ulcers after resumption of the use of their contaminated materials. In 1981, we had a similar episode of successional *Serratia* corneal ulcers with a patient who used a contaminated steroid suspension. Therefore, the clinician should emphasize the need for patients to discard used ocular steroid suspensions and contact lens solutions following any infectious episode.

The failure of patients to maintain good hygienic practices in the handling of their solutions appeared to underlie the development of most corneal ulcers. The resistance of bacteria to currently used preservatives further stresses the need of the patients to be meticulous in cleaning and disinfection procedures and the need of the physician to be alert to possible contamination of ocular medicants.

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