Jpn J Ophthalmol 2010;54:550–554
DOI 10.1007/s10384-010-0874-1
© Japanese Ophthalmological Society 2010

CLINICAL INVESTIGATION

Molecular Genetic Investigations of Contaminated Contact Lens Storage Cases as Reservoirs of *Pseudomonas aeruginosa* Keratitis

Yoko Ogushi[1], Hiroshi Eguchi[1], Tomomi Kuwahara[2], Naoko Hayabuchi[3], and Masako Kawabata[4]

[1] Department of Ophthalmology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan; [2] Department of Molecular Bacteriology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan; [3] Eye Clinic Josanjima, Tokushima, Japan; [4] Department of Ophthalmology, Anan Kyoei Hospital, Anan, Japan

Abstract

Purpose: To elucidate the reservoirs of *Pseudomonas aeruginosa* keratitis associated with contact lens (CL) wear by using a molecular genotyping method.

Method: Ten *P. aeruginosa* isolates obtained from two young adult patients with infectious keratitis associated with CL wear were studied. These were isolated from corneal specimens, conjunctival swabs, discharges, CL storage cases, and the living environment of the two patients. Species identification was performed with an Oxi/Ferm Tube II system using well-separated colonies on MacConkey and NAC agar plates. We employed molecular genotyping by pulsed-field gel electrophoresis.

Results: We isolated three (one each from a corneal scraping, discharge, and a CL storage case) *P. aeruginosa* samples from patient 1, and seven (one each from the conjunctival swab, CL storage case, and the patient's fingers, and four from the patient's room) from patient 2. *Pseudomonas aeruginosa* of environmental origin could not be obtained from the house of patient 1. The genotypes of two *P. aeruginosa* isolates, from corneal scraping and discharge, were identical to that of the isolate from the CL storage case belonging to patient 1. In patient 2, the isolates from the eye and the CL storage case showed the identical genotype, which was different from those of the environmental isolates.

Conclusion: Our results suggest that the causative *P. aeruginosa* strains in cases of infectious keratitis associated with CL wear originate in contaminated CL storage cases. However, it is still unclear whether the environmental strains in the patients' houses also cause keratitis. **Jpn J Ophthalmol** 2010;54:550–554 © Japanese Ophthalmological Society 2010

Keywords: contact lens storage case, environment, pulsed-field gel electrophoresis, *Pseudomonas aeruginosa* keratitis

Introduction

Microbial keratitis is a severe corneal infectious disease found among contact lens (CL) wearers, occasionally causing corneal melting or perforation with a high risk of visual loss. Of the bacterial causative agents of infectious keratitis, Gram-negative rods of *Pseudomonas* species have consistently been the most common isolates.[1–4] For this reason, *P. aeruginosa* is the most important species to be considered during diagnosis and treatment of bacterial keratitis. *Pseudomonas aeruginosa* is judged to be the causative pathogen of infectious keratitis when the bacterium is detected in infected corneal specimens because the species is not a commensal bacterium of the ocular surface. This

Received: January 13, 2010 / Accepted: June 29, 2010
Correspondence and reprint requests to: Hiroshi Eguchi, Department of Ophthalmology, Institute of Health Biosciences, The University of Tokushima Graduate School, 3-18-15 Kuramoto-cho, Tokushima 770-8503, Japan
e-mail: hiroegu@clin.med.tokushima-u.ac.jp

Table 1. Epidemiological data of the subjects ($n = 2$)

Patient	Age (years)	Sex	Contact lens type	Disinfectant	Hygienic care	Symptoms	Findings in anterior segments	BCVA of infected eye
1	23	Male	Biweekly disposable	MPS	Overnight wear Extended wear Not rubbing lens	Eye pain Conjunctival hyperemia Discharge Visual disturbances	Corneal abscess Corneal infiltration Conjunctival hyperemia Ciliary injection Hypopyon iritis	Hand motion
2	27	Male	Daily disposable	MPS	Overnight wear Extended wear Not rubbing lens Not changing MPS	Eye pain Conjunctival hyperemia Discharge Visual disturbances	Multiple corneal abscesses Corneal infiltration Conjunctival hyperemia Ciliary injection iritis	(0.01)

MPS, multipurpose solution; BCVA, best-corrected visual acuity.

species is also frequently detected in CL accessories, specifically in the CL storage cases of patients with bacterial keratitis associated with CL wear[5] and of asymptomatic CL wearers.[6,7]

However, there is little direct evidence demonstrating that the clinical *P. aeruginosa* isolates from corneal specimens originate from the patients' contaminated CL storage cases. Moreover, it has been argued that some soft-lens wearers may be infected by Gram-negative organisms from environmental sources rather than from contaminated lens care materials.[1] Since *P. aeruginosa* is a commonly encountered bacterium in humid environments and several unrelated *P. aeruginosa* strains can potentially contaminate CL storage cases simultaneously, it is difficult to distinguish the origin of each isolate by bacteriological culture examinations alone. The contamination route needs to be elucidated by molecular genetic techniques in cases caused by noncommensal pathogens in order to control the infections.

Molecular genotyping by pulsed-field gel electrophoresis (PFGE) is widely used for assessing nosocomial transmission of multidrug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus*[8] and multidrug-resistant *P. aeruginosa*.[9] It is a sensitive epidemiological tool that can produce discernible genomic fingerprints for elucidating pathogen transmission routes. In this method, restriction endonucleases digest chromosomal DNA embedded in low-melting agarose to produce high-molecular-weight DNA fragments. These large fragments are fractionated in an agarose gel by periodic switching of the electric field orientation across the gel. Isolates showing identical PFGE patterns are generally considered to be of the same origin.[10] This method is, therefore, effective for tracing routes of bacterial contamination.[11–15]

In this study, we examined the *P. aeruginosa* isolates from corneal specimens, conjunctival swabs and discharges, and CL storage cases of two patients with *P. aeruginosa* keratitis associated with CL wear and used PFGE to identify the possible transmission route of the bacterium to the ocular surface.

Materials and Methods

Patient Data

We examined two cases of *P. aeruginosa* keratitis associated with CL wear. Clinical information of the two patients is provided in Table 1. The patients were healthy young adults (23- and 27-year-old men) who wore disposable soft CLs. Both patients had a history of poor CL hygiene, extended wear, overnight wear, lack of CL washing, and repeated use of a multipurpose solution in their CL storage cases.

Bacterial Isolates

Scraped corneal tissues, conjunctival swabs (taken immediately after corneal scraping) and discharges, and multipurpose solutions in CL storage cases were submitted for microbiological examination. The samples were spread on sheep blood agar plates (Eiken Chemical, Tochigi, Japan), MacConkey agar plates (Eiken), and NAC agar plates (Eiken). Well-separated colonies of each isolate were selected for species identification and PFGE genotyping. One *P. aeruginosa* strain, isolated from a conjunctival swab from patient 2, was provided as a pure culture in a semisolid medium from the clinical laboratory of Anan Kyoei Hospital, Japan. Of these isolates, the colonies with positive oxidase test (Eiken) results were inoculated into an Oxi/Ferm Tube II (Becton Dickinson Japan, Tokyo, Japan). Species identification was based on ID profiling according to the manufacturer's instructions. Of the *P. aeruginosa* isolates from each patient, those showing the same ID profile were analyzed by PFGE.

Pulsed-Field Gel Electrophoresis

The *P. aeruginosa* isolates were inoculated in 3 ml of LB broth (Becton Dickinson, Sparks, NV, USA) and cultured

Jpn J Ophthalmol
Vol 54: 550–554, 2010

without shaking for 24–48 h at 37°C. PFGE plugs were prepared with the Gene Path Group 3 Kit (Bio-Rad Laboratories, Steenvoorde, France) according to the manufacturer's instructions. In brief, 1.0 ml of each culture was centrifuged at 12 000 *g* for 1 min. The pellets were suspended in 150 μl of cell suspension buffer and kept at 50°C. An equal volume (150 μl) of preheated 1.6% low-melting-point agarose at 50°C was added to each cell suspension. The mixture was poured into sample plug molds and solidified at 4°C for 30 min. The plugs were settled into new tubes and incubated in 500 μl of lysis buffer for 1 h at 37°C. After the lysis buffer was replaced with proteinase K buffer, 150 μl of proteinase K solution was added to the tubes, and the plugs were incubated for 20 h at 50°C. The plugs were then washed three times with 1× Tris-EDTA (TE) and once with 0.1× TE for 30 min at room temperature. After washing, the plugs were equilibrated with 300 μl of 1× *Spe*I buffer for 30 min. For restriction endonuclease digestion, the plugs were settled into new tubes containing 500 μl each of 1× *Spe*I buffer containing 8.3 μl of *Spe*I. The tubes were incubated for 16–20 h at 37°C. PFGE was performed with the CHEFF-DR II system (Bio-Rad). The digested plugs were incorporated into a 1.2% agarose gel and electrophoresed under the following conditions: run time, 30 h; buffer temperature, 14°C; electric field strength, 200 volts/cm; and linear ramping switch time, 5–30 s.

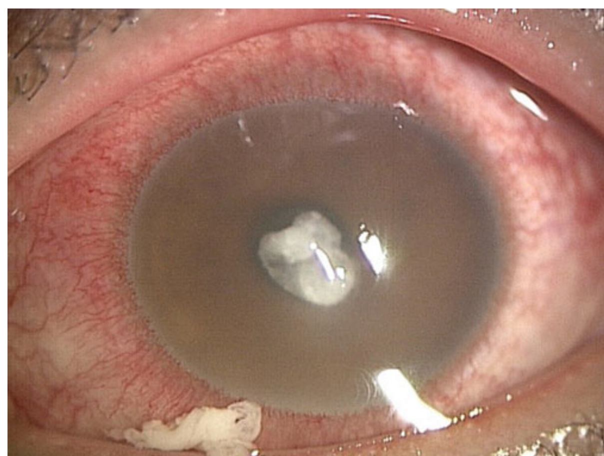

Figure 1. Clinical findings of *Pseudomonas aeruginosa* keratitis (patient 1). Marked conjunctival hyperemia, corneal abscess, diffuse corneal edema, whitish discharge, and a small amount of hypopyon were found in the left eye.

Results

Clinical Findings and Outcomes

Clinical information of the two patients is summarized in Table 1. In both patients, slit-lamp microscopy revealed severe conjunctival hyperemia and discharge, corneal edema, and corneal abscess (Figs. 1 and 2). Treatment at 2-h intervals with aminoglycoside plus quinolone ophthalmic solution for a month was effective in both patients. In patient 1, visual acuity OS increased (to 1.5) from the premedication acuity (hand motion). In patient 2, visual acuity OD also increased (to 1.0) from the premedication value (0.01). Only faint corneal opacities were found in the infected area 2 years (patient 1) and 6 months (patient 2) after the treatment.

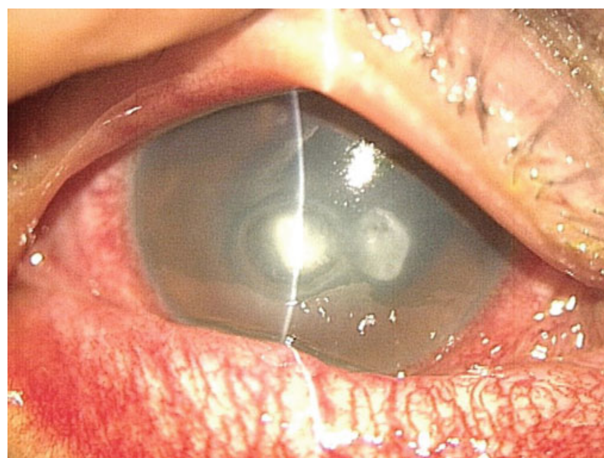

Figure 2. Clinical findings of *Pseudomonas aeruginosa* keratitis (patient 2). Marked conjunctival hyperemia, multiple corneal abscesses, and diffuse corneal edema were found in the right eye.

Microbiological Examination and Species Identification

The *Pseudomonas* isolates obtained in this study are listed in Table 2. From these two cases of *P. aeruginosa* keratitis, a total of ten *P. aeruginosa* isolates were obtained from corneal scrapings, conjunctival swabs, discharges, CL storage cases, patient fingers, and the home environment of one of the patients (underneath the CL storage cases, a humid area within a 1-m radius of the CL storage cases, the tap handle, and the balcony). In the case of patient 1, three isolates were obtained: from a corneal scraping, from discharge, and from a CL storage case. No *P. aeruginosa* could be isolated from the home environment of patient 1. Seven isolates were obtained from patient 2: one isolate each from a conjunctival swab and a CL storage case, one from the patient's fingers, and four from the home environment (underneath the CL storage cases, a tap handle, and the balcony). One *Pseudomonas* species other than *P. aeruginosa* was isolated from each patient. One *Pseudomonas* isolate that was obtained from the CL storage case of patient 1 could not be identified at the species level (ID profiling number 30303). One *P. putida* isolate was obtained from the CL storage case of patient 2.

Table 2. *Pseudomonas* isolates obtained in this study

Isolate no.	Patient	Sources	Species	ID profile[a]	PFGE type[b]
1	1	Corneal scraping	*P. aeruginosa*	30003	I
2	1	Discharge	*P. aeruginosa*	30003	I
3	1	CL storage case	*P. aeruginosa*	30003	I
4	2	Conjunctival swab	*P. aeruginosa*	30323	II
5	2	CL storage case	*P. aeruginosa*	30323	II
6	2	Underneath CL storage cases	*P. aeruginosa*	30323	III
7	2	Fingers	*P. aeruginosa*	30323	III
8	2	Humid area within a 1-meter radius from the CL storage case	*P. aeruginosa*	30323	IV
9	2	Tap handle	*P. aeruginosa*	30323	IV
10	2	Balcony	*P. aeruginosa*	30323	V
11	1	CL storage case	unidentified	30303	NT
12	2	CL storage case	*P. putida*	30307	NT

PFGE, pulsed-field gel electrophoresis; CL, contact lens; NT, not tested.
[a]ID profile numbers based on species identification with the Oxi/Ferm Tube II system are shown.
[b]PFGE type was classified based on the fragment patterns shown in Fig. 3.

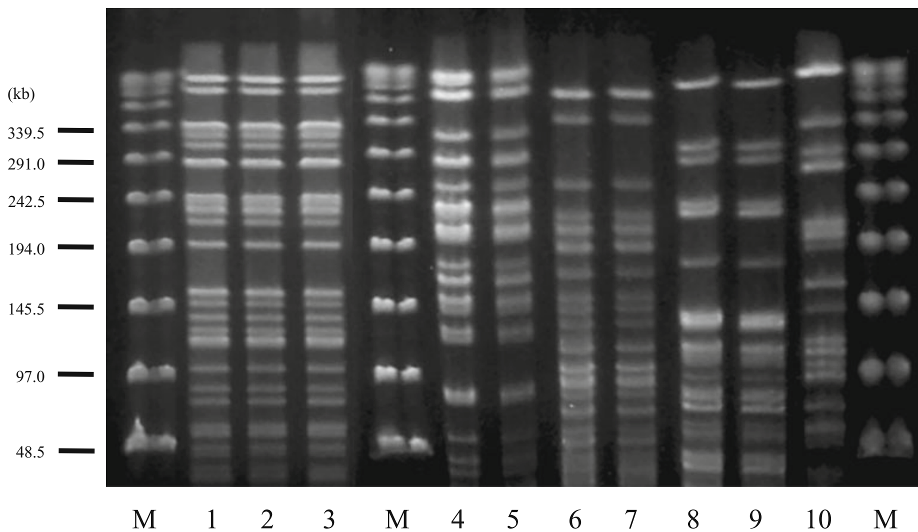

Figure 3. Pulsed-field gel electrophoresis (PGFE) results for ten *Pseudomonas aeruginosa* isolates from two patients. *Lanes 1, 2*, and *3* show the PGFE profiles of the isolates from a corneal scraping, discharge, and a contact lens (CL) storage case of patient 1, respectively. *Lanes 4* and *5* show the profiles of isolates from a conjunctival swab obtained just after corneal scraping and a CL storage case of patient 2, respectively. *Lanes 6–10* show the PFGE patterns of isolates from the home and finger of patient 2: *lane 6*, underneath CL storage cases; *lane 7*, finger; *lane 8*, a humid area within a 1-m radius of the CL storage case; *lane 9*, tap handle; and *lane 10*, balcony. *M*, lambda ladder PFGE markers (New England Bio Labs, Tokyo, Japan).

Molecular Genotyping of P. aeruginosa *Isolates by PFGE*

PFGE genotyping was performed on the ten *P. aeruginosa* isolates. The PFGE patterns of the isolates from the corneal scraping and discharge of patient 1 showed identical fragmentation patterns to those of the isolate from the CL storage case (lanes 1 to 3 in Fig. 3), indicating that these isolates were of the same origin. Similarly, the genotypes of the *P. aeruginosa* isolates from the conjunctival swab and the CL storage case of patient 2 were identical (lanes 4 and 5 in Fig. 3). On the other hand, an environmental *P. aeruginosa* isolate from patient 2's house (underneath the CL storage cases) showed an identical PFGE pattern to that of the isolate from his fingers (lanes 6 and 7 in Fig. 3). The PFGE patterns of the other two environmental isolates from the house of patient 2 (a humid area within a 1-m radius of the CL storage cases and tap handle) were identical to each other but different from those of the other isolates (lanes 8 and 9 in Fig. 3). None of the environmental isolates showed PFGE patterns identical to those of the isolates from the eye specimens. We could not isolate any environmental *P. aeruginosa* from the house of patient 1.

Discussion

Pseudomonas aeruginosa is the most important etiologic agent for bacterial keratitis, especially among CL wearers. Contaminated CL care solutions in storage cases due to poor lens or case hygiene may preserve the pathogens of keratitis. However, there is little molecular genetic evidence that *P. aeruginosa* isolated from infected ocular surfaces originates from contaminated CL care solutions in CL storage cases. The lack of evidence is due to the lack of convenient methods to trace and differentiate bacterial isolates at the strain level. To our knowledge, there has been only one case reported in the literature[16] in which several *P.*

aeruginosa isolates from a patient with bacterial keratitis associated with CL wear was analyzed by PFGE.

In most clinical laboratories microbial examination is usually performed at the species level. Although strain differentiation is rarely performed on bacterial isolates in a clinical setting, molecular genotyping has been effectively used to determine the epidemiology of infectious diseases and to identify nosocomial infection transmission routes. Among molecular genotyping methods for bacteria, PFGE is considered to be the gold standard for strain differentiation. Hence, we conducted this molecular epidemiology study using PFGE. Consistent with the report by de Melo et al.,[16] we demonstrated in two infectious keratitis patients that the *P. aeruginosa* isolates from the infectious ocular surfaces and the CL storage cases were of the same origin. This result indicates that the contaminated CL storage cases are reservoirs of the *P. aeruginosa* responsible for the keratitis associated with CL wear. Thus, microbial examination of CL storage cases and antimicrobial susceptibility tests of the obtained isolates would be valuable for clinical diagnosis and treatment of infectious keratitis cases when no significant isolates have been obtained from the ocular surface.

Furthermore, our study investigated for the first time the relationship of pathogenic strains of *P. aeruginosa* isolated from both ocular samples and a patient's living environment. Our results showed that *P. aeruginosa* contaminating CL storage cases was unlikely to have originated from the living environment of the patients. As shown in Fig. 3, the genotypes of the *P. aeruginosa* isolates from the corneal specimens of patient 2 were different from those of the environmental isolates, whereas the *P. aeruginosa* isolate from the patient's fingers showed the identical fragmentation pattern to that of the isolate from underneath the CL storage case. Since most CL wearers touch their CL storage cases during lens care, hand contact may be one transmission route, especially since transient colonization of the hands with *P. aeruginosa* is sufficient to initiate nosocomial infection.[17] However, whether *P. aeruginosa* isolates from ocular specimens in *P. aeruginosa* keratitis associated with CL wear originate in the living environment remains unresolved. Our results do not demonstrate a definite causal relationship between isolates from ocular specimens and from the patient's environment. Further similar molecular genotyping studies and the accumulation of more isolates are essential to reveal the role of environmental isolates of *P. aeruginosa* in infectious keratitis cases associated with CL wear. Although a large number of *P. aeruginosa* isolates from both corneal specimens and the living environment are needed, we expect that such data will provide useful information for the prevention of *P. aeruginosa* keratitis associated with CL wear by improving CL care strategies.

In conclusion, we demonstrated via PFGE that the *P. aeruginosa* strains in two infectious keratitis cases associated with CL wear originated from contaminated CL storage cases. The contaminants were possibly transmitted via the patient's hands during CL care. Accumulation of genotyping data from a large number of isolates, from both corneal specimens in *P. aeruginosa* keratitis and the living environments of patients, may elucidate further the transmission routes and origin of the contamination of CL storage cases by *P. aeruginosa* and aid in establishing a regimen for precautions against infectious keratitis associated with CL wear.

Acknowledgments. We thank Ms. Mayumi Miyazaki and Ms. Mariko Murakami, medical technologists affiliated with the Department of Clinical Laboratory in Anan Kyoei Hospital, for providing a strain of *Pseudomonas aeruginosa*, isolated from a conjunctival swab of patient 2 at Anan Kyoei Hospital.

References

1. Dart JKG. Predisposing factors in microbial keratitis: the significance of contact lens wear. Br J Ophthalmol 1988;72:926–930.
2. Gelentine PG, Cohen EJ, Laibson PR, et al. Corneal ulcers associated with contact lens wear. Arch Ophthalmol 1984;102:891–894.
3. Schein OD, Ormerod LD, Barraquer E, et al. Microbiology of contact lens-related ulcers. Cornea 1989;8:281–285.
4. Cheng KH, Leung SL, Hoekman HW, et al. Incidence of contact-lens-associated microbial keratitis and its related morbidity. Lancet 1999;354:181–185.
5. Devonshire P, Munro FA, Abernethy C, et al. Microbial contamination of contact lens cases in the west Scotland. Br J Ophthalmol 1993;77:41–45.
6. Houang E, Lam D, Fan D, et al. Microbial keratitis in Hong Kong: relationship to climate, environment and contact-lens disinfection. Trans R Soc Trop Med Hyg 2001;95:361–367.
7. Stapleton F, Dart JKG, Seal DV, et al. Epidemiology of *Pseudomonas aeruginosa* keratitis in contact lens wearers. Epidemiol Infect 1995;114:395–402.
8. Del'Alamo L, d'Azevedo PA, Strob AJ, et al. An outbreak of catalase-negative methicillin-resistant *Staphylococcus aureus*. J Hosp Infect 2007;65:226–230.
9. Cao B, Wang H, Sun H, et al. Risk factors and clinical outcomes of nosocomial multi-drug resistant *Pseudomonas aeruginosa* infections. J Hosp Infect 2004;57:112–118.
10. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995;33:2233–2239.
11. Kirschke DL, Jones TF, Craig AS, et al. *Pseudomonas aeruginosa* and *Serratia marcescens* contamination associated with a manufacturing defect in bronchoscopes. N Engl J Med 2003;348:214–220.
12. Miranda G, Kelly C, Solorzano F, et al. Use of pulsed-field gel electrophoresis typing to study an outbreak of infection due to *Serratia marcescens* in a neonatal intensive care unit. J Clin Microbiol 1996;34:3138–3141.
13. Srinivasan A, Wolfenden LL, Song X, et al. An outbreak of *Pseudomonas aeruginosa* infection associated with flexible bronchoscopes. N Engl J Med 2003;348:221–227.
14. Shimono N, Takuma T, Tsuchimochi N, et al. An outbreak of *Pseudomonas aeruginosa* infection following thoracic surgeries occurring via contamination of bronchoscopes and an automatic endoscope reprocessor. J Infect Chemother 2008;14:418–423.
15. DiazGranados CA, Jones MY, Kongphet-Tran T, et al. Outbreak of *Pseudomonas aeruginosa* infection associated with contamination of flexible bronchoscope. Infect Control Hosp Epidemiol 2009; 30:550–555.
16. de Melo GB, Aggio FB, Hofling-Lima AL, et al. Pulsed-field gel electrophoresis in the identification of the origin of bacterial keratitis caused by *Pseudomonas aeruginosa*. Graefes Arch Clin Exp Ophthalmol 2007;245:1053–1054.
17. Foca M, Jakob K, Whittier S, et al. Endemic *Pseudomonas aeruginosa* infection in a neonatal intensive care unit. N Engl J Med 2000;343:695–700.