CASE REPORTS

Two Cases of Keratitis and Corneal Ulcers Caused by *Burkholderia gladioli*[▽]

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We report two cases of protracted keratitis complicated by corneal ulcer. *Burkholderia gladioli*, primarily known as a plant pathogen, was cultured from corneal swabs. The epithelial defects healed after extended adequate antibiotic therapy. Despite identical patterns of both strains in restriction fragment length polymorphism analysis, a common infection source was not detectable.

CASE REPORTS

Case 1. An 18-year-old male presented at the outpatient clinics of the Ophthalmology Department in June 2006 with a foreign-body feeling in the right eye that had persisted for 2 weeks. He did not report any previous ocular trauma or ocular surface disease. Until the episode, he had not worn contact lenses or undergone eye surgery. In agreement with the local symptoms, his vision was slightly diminished on the right side. The best corrected visual acuity (BCVA) was 12/20 in the right eye and 24/20 in the left eye. To treat a suspected herpesvirus keratitis, several attempts at therapy involving local application of acyclovir, ofloxacin, and dexamethasone were performed, temporally accompanied by orally administered acyclovir (800 mg five times daily). In September 2006 he presented with an acute worsening of the symptoms. He complained of heavy eye pain, eyelid edema, and a diminished vision of the right eye (BCVA of the right eye, 1/40).

On examination an eyelid edema, mixed conjunctival injection, chemosis, infiltration of the corneal stroma with a central annular ulcer, edema of the epithelium, and beginning vascularization of the lower limbal area (Fig. 1) were recorded. There were no pathological findings for the contralateral eye.

The superficial corneal swab of the right eye was used for several microbiological tests. Herpesvirus infection was ruled out by a negative Herpes Consensus PCR (Argene Biosoft, Varilhes, France), which simultaneously amplifies targets from herpes simplex virus types 1 and 2, varicella-zoster virus, cytomegalovirus, Epstein-Barr virus, and human herpesvirus 6. Inoculated sheep blood, chocolate, and MacConkey agar plates exclusively grew gram-negative rods when aerobically cultured at 35°C for 7 days. The isolate was identified as *Burkholderia gladioli* by using the

API 20NE biochemical assay for nonfermentative gram-negative rods (bioMérieux, Nürtingen, Germany; code 0067577). The species identification was confirmed by sequencing the 16S rRNA gene region of the bacterial genome.

Antibiotic susceptibility testing was performed with Etest strips (AB Biodisk, Solna, Sweden) on Mueller-Hinton agar and interpreted according to the CLSI criteria (6) for systemically applied antibiotics (Table 1). When applying the results of the resistance testing to the situation in the patient's eye, one has to be aware that corneal infections are commonly treated with topically applied antibiotics at concentrations definitely above standard concentrations reached after systemic drug administration. However, testing of MICs and subsequent judgment of a bacterial strain's susceptibility address systemic drug concentrations.

Additional 18S rRNA gene-specific PCR from the swab of the right eye cornea led to detection of *Candida* spp. Since the yeast cells could not be cultured on the blood-containing media, it is probable that the cell load in the swab was low, indicating a secondary contamination. However, a mixed infection of the fungus and *B. gladioli* was formally possible.

According to these results the acyclovir therapy was stopped and the patient received a local therapy with 3 mg/ml ofloxacin. The antibiotic eyedrops were initially administered at hourly intervals. The medication exclusively directed to the bacterial isolate was continued for 6 weeks, during which time the ulcer slowly healed. The BCVA of the affected eye improved to 16/20 in December 2006 after correction by a contact lens.

Case 2. In December 2006 a 70-year-old man presented at the Department of Ophthalmology of the Rostock University Hospital with a 3-week history of redness and pain of the right eye and decreased vision. For a suspected herpes simplex virus keratitis, he had received local therapy with ofloxacin and acyclovir (three times daily) by an ambulant ophthalmologist. The medication did not lead to an improvement.

Upon admission, the patient reported recurrent keratitis for about 20 years, beginning with an ocular trauma of the right eye during gardening. He denied previous eye surgery or wearing contact lenses. Additionally, he had a history of arterial hypertension.

On clinical examination, the patient presented a right eye with an eyelid edema, slight chemosis, and remarkable injec-

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FIG. 1. Patient 1 on admission in September 2006 with keratitis and a central corneal ulcer.

tion. There was an ulcer reaching from the temporal lower quadrant to the center of the cornea, which was positive upon fluorescence staining (Fig. 2). Additionally, the cornea displayed circular vascularization. There was a mild sign of intraocular inflammation with positive Tyndall phenomenon, cells in the anterior chamber, and a hyperemic iris with a posterior synechia at 4 o'clock. The fundus on both sides and the whole left eye were without pathological findings. The sensibility of the cornea was intact, and sensibilities on both

sides were equal. The vision was diminished on the right eye (BCVA of the right and left eyes, 6/20 and 20/20, respectively).

A swab from the corneal ulcer was subjected to the same analytical procedures as before. Herpesvirus-specific PCR as well as herpesvirus serology remained negative, making a herpesvirus infection very unlikely. Again, from the three agar media pure cultures of *Burkholderia gladioli* were grown. The bacterial isolate was differentiated to species level by testing a biochemical profile (API 20NE; bioMérieux; code 0067577) and by 16S rRNA gene sequencing.

According to the results from susceptibility testing the patient received levofloxacin (5 mg/ml) locally administered every hour and supplemented by short-acting mydriatics and atropine. To support the wound healing, serum eyedrops were given. This medication was prepared in the University Hospital pharmacy by mixing the patient's serum and isotonic sodium chloride solution at a 1:1 ratio. The patient slowly improved and was discharged after 2 weeks with a BCVA of 6/20.

Two months later (February 2007) his condition worsened again. There was no healing of the epithelial defect. A tarsorrhaphy was performed, and the sutures applied during the procedure remained in place for 2 months. The antibiotic therapy was continued until March 2007. The serum medication of eyedrops was administered until September 2007. Finally, the epithelial defect closed with a persistent vascularized scar. The final BCVA at the last presentation of the patient was 12/20.

TABLE 1. Antimicrobial susceptibility profiles for the *Burkholderia gladioli* strains from the two reported patients

Antimicrobial agent	MIC (mg/liter) (interpretation) ^a for patient:	
	1	2
Piperacillin	2 (S)	2 (S)
Piperacillin-tazobactam	0.75 (S)	1 (S)
Cefotaxime	>32 (R)	>32 (R)
Ceftazidime	16 (I)	16 (I)
Ciprofloxacin	0.25 (S)	0.38 (S)
Levofloxacin	0.5 (S)	0.5 (S)
Imipenem	0.19 (S)	0.25 (S)
Meropenem	1.0 (S)	0.75 (S)
Gentamicin	0.38 (S)	0.5 (S)
Tobramycin	0.25 (S)	0.38 (S)
Trimethoprim-sulfamethoxazole	0.19/38 (S)	0.094/38 (S)
Tetracycline	12 (I)	12 (I)

^a MICs were determined by Etest (AB Biodisk, Sweden). Interpretation was according to CLSI standards. S, susceptible; I, intermediate; R, resistant. Values separated by slashes represent results for trimethoprim and for sulfamethoxazole, respectively.

No other case of *B. gladioli* keratitis has been recorded in the

FIG. 2. Patient 2 with keratitis and corneal ulcer of the temporal lower quadrant.

Rostock University Hospital from 1998 to the present. Since the Ophthalmology Department of the University Hospital is the only specific clinic in a 90-km radius, severe cases of keratitis in this area will present there. Hence, it is highly probable that no other case of *B. gladioli* keratitis happened during the observation period.

According to the literature, *B. gladioli* eye infections are extremely rare events (see below). Thus, the two described cases within only 1 year prompted us to examine the epidemiologic background and the potential relatedness of both cases.

The two *B. gladioli* patient isolates were subjected to SpeI restriction fragment length polymorphism analysis by pulsed-field gel electrophoresis. The two isolates displayed identical band patterns (data not shown). However, the discriminatory power of restriction fragment length polymorphism analysis specifically for *B. gladioli* has not been studied so far. Thus, it is formally possible that a clone of *B. gladioli* is widely distributed in the local area and therefore may affect persons at considerable distances.

Specimens were taken from both patients within the first 24 h after admission to the hospital, which by definition excludes a nosocomial infection. A thorough anamnesis of both patients did not reveal any opportunity for contact between both patients in their private lives or during their presence in the Ophthalmology Department. Before presenting to the University Hospital, both patients were seen by different private ophthalmologic practices. During their therapy, on some occasions the patients were examined in the same rooms of the outpatient clinics, although the examinations were at least 4 months apart. Neither patient underwent tonometry, and all

instruments with direct eye contact were properly processed in the Central Sterilization Unit of the University Hospital according to approved standard operation procedures. Personal contact lenses used by both patients were always stored directly at the patients' beds, with individual reservoirs of lens cleaning and storage fluids.

In February 2007, the tap water in all examination rooms of the ophthalmologic outpatient clinic was tested in the accredited Microbiology and Hygiene Diagnostics Laboratories of the University Hospital. In parallel, swabs from the corresponding sinks as well as from every fluid reservoir in the examination rooms were selectively screened for the presence of *B. gladioli*. The bacteria could not be detected in any tested specimen.

The worldwide annual incidence of infectious keratitis is estimated to vary from 6 per 100,000 inhabitants in Hong Kong to 710 per 100,000 inhabitants in Burma (12, 15, 24). To our knowledge there is no reliable epidemiological data about keratitis in Germany. In the Rostock University Hospital, which serves roughly 500,000 regional inhabitants, between 10 and 20 cases of infectious keratitis cases are seen per year.

The causative agents of corneal ulcers differ widely in various countries and regions. In about 35 to 60% of patients with suspected infective keratitis the involved pathogens cannot be identified (24). In Paraguay and India, gram-negative bacteria are commonly isolated. In Japan and other industrialized countries, gram-positive bacteria such as coagulase-negative staphylococci predominate (25). Yet, the incidence of gram-negative rods also increases in these regions of the world (18). Herpetic keratitis was found in about 7% of the cases (12).

Wearing contact lenses is a major risk factor for infectious keratitis: in up to 50% of the cases described, patients wore them. In this subgroup of patients, infections with nonfermenters like *Pseudomonas aeruginosa* and free-living amoebas like *Acanthamoeba* spp. are more common. Also, agricultural work and outdoor occupations appear to predispose to infectious keratitis, most probably due to contact with infectious dust, vapor, or water plus an increased risk of mechanical damage to the cornea.

Burkholderia gladioli is a plant pathogen, associated with diseases of the gladiolus plant and decaying onions, and is mainly found in soil, in environmental water, and on plants and fruits (16). It is a nonfermentative gram-negative bacillus, closely related to the *Pseudomonas* genus. Recently, *Pseudomonas* rRNA homology group II was reclassified to the genus *Burkholderia* (27). At this occasion, also the species *B. gladioli* was defined. In 2003, Jiao et al. described the cocovenenans pathovar of *B. gladioli*, which appears to be responsible for the majority of human infection cases (10). Other *Burkholderia* species are well known as obligate or facultative human pathogens like *B. mallei*, causing glanders, *B. pseudomallei*, causing melioidosis, and *B. cepacia*, causing respiratory tract infections in cystic fibrosis patients.

B. gladioli itself is a rare, opportunistic human pathogen, most commonly reported in lung infections of patients with cystic fibrosis. These episodes are occasionally complicated by recurrent abscesses or sepsis (1, 2, 3, 4, 7, 11, 13, 14, 22, 26). Ross et al. reported pneumonia in two patients with chronic granulomatosis (20). The bacterium was rarely isolated in cases of sepsis similar to melioidosis, osteomyelitis in patients with AIDS, and chronic granulomatosis, as well as in a case of fever after liver transplantation (5, 8, 9, 21, 23).

According to NCBI data bank searches using “*Burkholderia gladioli*” or “*Pseudomonas* AND keratitis” as search terms, only one definite case of an ocular infection with *B. gladioli* was described by Ritterband et al. in 2002 (19). Due to changes in the nomenclature, it is impossible to decide whether the first case was reported by Mahajan et al. in 1982 (17). The patient of Ritterband et al. had had a corneal graft for about 1 year when he developed recurrent keratitis and consecutive endophthalmitis caused by a *B. gladioli* infection. The infection took a complicated course despite of an intravitreal antibiotic therapy with vancomycin and ceftazidime combined with a topical therapy with ciprofloxacin over 4 months.

Since almost all of the reported systemic cases occurred in severely immunocompromised persons, *B. gladioli* seems to have a low pathogenic potential. *B. gladioli* can easily be neutralized by human serum or complement factors. This mechanism apparently protects most healthy individuals from an infection (11). Patients with complement deficiencies or an impaired phagocytotic function are at high risk of developing a symptomatic infection (11, 20). The absence of protective serum factors in the local compartment of the eye explains why *B. gladioli* infection could develop in spite of a healthy immune response or the absence of underlying diseases.

Probably, ocular infections with *B. gladioli* are more common than the scarcity of publications in the current databases suggests. The two obviously independent cases in the present report underscore this hypothesis and could even indicate an increasing incidence of the pathogen. In addition, the cases

should stimulate the awareness of ophthalmologists and laboratory staff to identify common yet undetected sources in the patients' environment. Before 1997, commercial identification systems could not discriminate the species from the related species *B. cepacia* (8), implying incorrect identification of *B. gladioli* strains. After that year, differentiation solely based on biochemical profiles could be misleading and, therefore, confirmation of species diagnosis should be made by sequencing the 16S rRNA gene complexes.

Now, the three reported cases of ocular infections by *B. gladioli* point out the pathogenic potential of this species, which can obviously cause keratitis, corneal ulcer, and endophthalmitis in immunocompetent persons. According to the experience with therapy in cystic fibrosis patients, *B. gladioli* is most susceptible to quinolones, aminoglycosides, and imipenem, while ampicillin, cephalosporins, and aztreonam are commonly not effective (8). An early diagnosis based on solid microbiologic data plus a specific antibiotic therapy which should use topically administered quinolones, potentially in combination with the systemically applied drug, appear to be essential to prevent a protracted clinical course and severe complications.

REFERENCES

1. Alice, T., S. Scutera, M. G. Chirillo, and D. Savoia. 2006. *Burkholderia* respiratory tract infections in Italian patients with cystic fibrosis: molecular characterization. *J. Infect.* **53**:159–165.
2. Barker, P. M., R. E. Wood, and P. H. Gilligan. 1997. Lung infection with *Burkholderia gladioli* in a child with cystic fibrosis: acute clinical and spirometric deterioration. *Pediatr. Pulmonol.* **23**:123–125.
3. Bauernfeind, A., I. Schneider, R. Jungwirth, and C. Røller. 1998. Discrimination of *Burkholderia gladioli* from other *Burkholderia* species detectable in cystic fibrosis patients by PCR. *J. Clin. Microbiol.* **36**:2748–2751.
4. Baxter, I. A., P. A. Lambert, and I. N. Simpson. 1997. Isolation from clinical sources of *Burkholderia cepacia* possessing characteristics of *Burkholderia gladioli*. *J. Antimicrob. Chemother.* **39**:169–175.
5. Boyanton, B. L., Jr., L. M. Noroski, H. Reddy, M. K. Dishop, and M. J. Hicks. 2005. *Burkholderia gladioli* osteomyelitis in association with chronic granulomatous disease: case report and review. *Pediatr. Infect. Dis.* **24**:837–839.
6. Clinical and Laboratory Standards Institute. 2006. Performance standards for antimicrobial susceptibility testing; supplement M100-S16. Clinical and Laboratory Standards Institute, Wayne, PA.
7. Clode, F. E., L. A. Metherell, and T. L. Pitt. 1999. Nosocomial acquisition of *Burkholderia gladioli* in patients with cystic fibrosis. *Am. J. Respir. Crit. Care Med.* **160**:374–375.
8. Graves, M., T. Robin, A. M. Chipman, J. Wong, S. Kashe, and J. M. Janda. 1997. Four additional cases of *Burkholderia gladioli* infection with microbiological correlates and review. *Clin. Infect. Dis.* **25**:838–842.
9. Hoare, S., and A. J. Cant. 1996. Chronic granulomatous disease presenting as severe sepsis due to *Burkholderia gladioli*. *Clin. Infect. Dis.* **23**:411.
10. Jiao, Z., Y. Kawamura, N. Mishima, R. Yang, N. Li, X. Liu, and T. Ezaki. 2003. Need to differentiate lethal toxin-producing strains of *Burkholderia gladioli*, which cause severe food poisoning: description of *B. gladioli* pathovar cocovenenans and an emended description of *B. gladioli*. *Microbiol. Immunol.* **47**:915–925.
11. Jones, A. M., T. N. Stanbridge, B. J. Isalska, M. E. Dodd, and A. K. Webb. 2001. *Burkholderia gladioli*: recurrent abscesses in a patient with cystic fibrosis. *J. Infect.* **42**:69–71.
12. Keay, L., K. Edwards, T. Naduvilath, H. R. Taylor, G. R. Snibson, K. Forde, and F. Stapleton. 2006. Microbial keratitis: predisposing factors and morbidity. *Ophthalmology* **113**:109–116.
13. Kennedy, M. P., R. D. Coakley, S. H. Donaldson, R. M. Aris, K. Hohnacker, J. P. Wedd, M. R. Knowles, P. H. Gilligan, and J. R. Yankaskas. 2007. *Burkholderia gladioli*: five year experience in a cystic fibrosis and lung transplantation centre. *J. Cyst. Fibros.* **6**:267–273.
14. Khan, S. U., S. M. Gordon, P. C. Stillwell, T. J. Kirby, and A. C. Arroliga. 1996. Empyema and bloodstream infection caused by *Burkholderia gladioli* in a patient with cystic fibrosis after lung transplantation. *Pediatr. Infect. Dis. J.* **15**:637–639.
15. Lam, D. S., E. Houang, D. S. Fan, D. Lyon, D. Seal, and E. Wong for the Hong Kong Microbial Keratitis Study Group. 2002. Incidence and risk factors for microbial keratitis in Hong Kong: comparison with Europe and North America. *Eye* **16**:608–618.

16. LiPuma, J. J., B. J. Currie, G. D. Lum, and P. A. R. Vandamme. 2007. *Burkholderia*, *Stenotrophomonas*, *Ralstonia*, *Cupriavidus*, *Pandoraea*, *Brevundimonas*, *Comamonas*, *Delftia*, and *Acidovorax*, p. 749–769. In P. R. Murray, E. J. Baron, J. H. Jorgensen, M. L. Landry, and M. A. Pfaller (ed.), *Manual of clinical microbiology*, 9th ed. ASM Press, Washington, DC.
17. Mahajan, V. M., T. A. Srikanth, L. P. Agarwal, and Shriniwas. 1982. *Pseudomonas aeruginosa*: isolation from ocular tissues. Aeruginocin typing and its relationship to corneal pathogenicity in rabbits. *Int. Ophthalmol.* **4**:169–176.
18. Pleyer, U., and W. Behrens-Baumann. 2007. Bacterial keratitis. Current diagnostic aspects. *Ophthalmologe* **104**:9–14.
19. Ritterband, D., M. Shah, K. Cohen, L. Lawrence, and J. Sedor. 2002. *Burkholderia gladioli* keratitis associated with consecutive recurrent endophthalmitis. *Cornea* **21**:602–603.
20. Ross, J. P., S. M. Holland, V. J. Gill, E. S. DeCarlo, and J. I. Gallin. 1995. Severe *Burkholderia (Pseudomonas) gladioli* infection in chronic granulomatous disease: report of two successfully treated cases. *Clin. Infect. Dis.* **21**:1291–1293.
21. Shin, J. H., S. H. Kim, M. G. Shin, S. P. Suh, D. W. Ryang, and M. H. Jeong. 1997. Bacteremia due to *Burkholderia gladioli*: case report. *Clin. Infect. Dis.* **25**:1264–1265.
22. Simpson, I. N., J. Finlay, D. J. Winstanley, N. Dewhurst, J. W. Nelson, S. L. Butler, and J. R. Govan. 1994. Multi-resistance isolates possessing characteristics of both *Burkholderia (Pseudomonas) cepacia* and *Burkholderia gladioli* from patients with cystic fibrosis. *J. Antimicrob. Chemother.* **34**:353–361.
23. Sudendey, J., E. Halle, A. Moter, L. Morawietz, and H. Olze. 2007. Melioidosis-like disease: infection with *Burkholderia coccovenenans* as rare differential diagnosis for lymphadenitis colli. *Laryngorhinootologie* **86**:287–290.
24. Thomas, P. A., and P. Geraldine. 2007. Infectious keratitis. *Curr. Opin. Infect. Dis.* **20**:129–142.
25. Toshida, H., N. Kogure, N. Inoue, and A. Murakami. 2007. Trends in microbial keratitis in Japan. *Eye Contact Lens* **33**:70–73.
26. Wilsher, M. L., J. Kolbe, A. J. Morris, and D. F. Welch. 1997. Nosocomial acquisition of *Burkholderia gladioli* in patients with cystic fibrosis. *Am. J. Respir. Crit. Care Med.* **155**:1436–1440.
27. Yabuuchi, E., Y. Kosako, H. Oyaizu, I. Yano, H. Hotta, Y. Hashimoto, T. Ezaki, and M. Arakawa. 1992. Proposal of *Burkholderia* gen. nov. and transfer of seven species of the *Pseudomonas* homology group II to the new genus, with the type species *Burkholderia cepacia* (Paleroni and Holmes 1981) comb. nov. *Microbiol. Immunol.* **36**:1251–1275.