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CLINICAL INVESTIGATION

Moraxella species: infectious microbes identified by use of time-of-flight mass spectrometry

Shunsuke Takahashi[1] · Kazuhiro Murata[1] · Kenji Ozawa[1] · Hiroki Yamada[2] · Hideaki Kawakami[3] · Asami Nakayama[4] · Yuko Asano[5] · Kiyofumi Mochizuki[1] · Hiroshige Mikamo[6]

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Abstract

Purpose To report the clinical manifestations, identification, antimicrobial susceptibilities, and treatment outcomes of ocular infections caused by *Moraxella* species.

Study design Retrospective study.

Patients and methods The medical records of all patients treated at the Departments of Ophthalmology of the Ogaki Municipal Hospital and the Gifu University Graduate School of Medicine for ocular infections caused by *Moraxella* species between January 2011 and June 2017 were examined. The stored *Moraxella* species isolated from ocular samples were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), molecular identification, and the biochemical properties.

Results Sixteen eyes of 16 patients were treated for *Moraxella* ocular infections. The patients' median age was 72 years. A predisposing systemic or ocular condition was identified in 15 of the patients. Nine of the patients developed keratitis; four, conjunctivitis; and three, blebitis. *M lacunata* (6 eyes), *M catarrhalis* (6), *M nonliquefaciens* (3), and *M osloensis* (1) were identified by MALDI-TOF MS. All isolates were sensitive to levofloxacin, tobramycin, ceftazidime, ceftriaxone, and cefazolin. Twelve patients with keratitis or blebitis were treated with various topical antimicrobial combinations, and systemic antibiotics were used in 10 of the 12 patients. The mean time for the complete closure of the epithelial defects with keratitis was 24 days. The visual outcomes after treatment were favorable except in 1 keratitis patient who underwent enucleation.

Conclusions The use of duo-therapy with a combination of fluoroquinolone and cefmenoxime should be considered in cases nonresponsive to monotherapy, such as keratitis and bleb-associated infections. MALDI-TOF MS is useful for the identification of *Moraxella* to the species level.

Keywords Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry · *Moraxella* species · Ocular infection

Introduction

Moraxella species are gram-negative bacilli, or coccobacilli. They are strictly aerobic, oxidase-positive, catalase-positive, DNAse-positive, nonencapsulated, and asaccharolytic. The

Corresponding author: Hideaki Kawakami

✉ Hideaki Kawakami
hkawa-gif@umin.net

[1] Department of Ophthalmology, Gifu University Graduate School of Medicine, Gifu, Japan

[2] Department of Ophthalmology, Ogaki Municipal Hospital, Gifu, Japan

[3] Department of Ophthalmology, Gifu Municipal Hospital, 7-1 Kashima-cho, Gifu-shi, Gifu 500-8513, Japan

[4] Department of Clinical Laboratory, Gifu University Graduate School of Medicine, Gifu, Japan

[5] Department of Clinical Laboratory, Ogaki Municipal Hospital, Gifu, Japan

[6] Department of Clinical Infectious Diseases, Aichi Medical University, Aichi, Japan

Moraxella species are commensals of the nasopharynx and are a human pathogen that can cause otitis media, sinusitis, and respiratory tract infections. In the eye, *Moraxella* species have been reported to cause conjunctivitis, keratitis, blepharoconjunctivitis, and endophthalmitis [1–6].

Among the *Moraxella* species, *M catarrhalis* is the most clinically important, and it is one of the most common causative organisms of bacterial conjunctivitis in infants and older children [7]. Other than *M catarrhalis*, the *Moraxella* species are generally considered to have low pathogenic potential and account for approximately 5% of all corneal ulcers [8]. *Moraxella* keratitis can lead to severe ulcerations and is reported to be associated with ocular and systemic risk factors for severe vision reduction such as diabetes and chronic alcoholism [3, 4]. Cases of bleb-related delayed-onset endophthalmitis that were caused by *Moraxella* species have been reported [1, 3, 9, 10]. Also, the detection rate of *M catarrhalis* was reported to increase after natural disasters, such as tsunami, especially in flooded areas. It was reported that the detection rates of *M catarrhalis* increased from 0–3.8% before the Great East Japan Earthquake of 2011 to 31.3% (26/83) after it ($P < .01$) [11]. *M catarrhalis* was isolated widely from evacuees living in shelters and from persons living at home without running water and/or electricity [11]. Thus, at the time of a disaster, it is important to pay attention to infections related not only to pneumonia but also to keratitis.

Most laboratories cannot identify *Moraxella* species other than *M catarrhalis* because the colony morphology changes depending on the culture medium used and differs among the strains of *Moraxella* species. Most *Moraxella* species have similar biochemical responses. The technique for isolating *Moraxella* species has remained consistent over the past years. Recently, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has become a tool for rapid, accurate, and cost-effective identification of cultured bacteria and fungi based on automated analysis of the mass distribution of their proteins [12].

The purpose of this study was to determine the identification by MALDI-TOF MS and antimicrobial susceptibilities of *Moraxella* species, as well as the clinical manifestations and treatment outcomes of ocular infections caused by the species.

Patients and methods

Patients

The study protocol was approved by the institutional review boards of Gifu University Graduate School of Medicine and Ogaki Municipal Hospital. We reviewed the medical records of all patients treated at the Departments of Ophthalmology of the 2 institutions from January 2011 through June 2017 for ocular infections caused by *Moraxella* species.

The medical records of all the patients who received a diagnosis of *Moraxella*-associated disease were reviewed, including the age, sex, laterality, presenting complaints, history of topical steroid use, cultured species, antimicrobial susceptibilities, treatment methods, and initial and final best-corrected visual acuities (BCVAs). The main outcome measure was the BCVA, and a BCVA of $\leq$ 1.0 logMAR units was taken to be a good visual outcome [13]. Other outcome measures included the results of microbiologic investigations and the anatomic and clinical outcomes.

Microbiologic tests

All specimens other than *M catarrhalis* were identified to the level of *Moraxella* genus by the colony and microscopic examinations (Gram staining and biochemical testing) and stored at each institute. All the stored specimens were cultured on 5% blood sheep blood agar and chocolate agar plates at 35°C for 24 to 72 h and were later identified to the species level simultaneously by the ID-test HN-20 Rapid system (Nissui) and MALDI-TOF MS. The MALDI-TOF MS identifications were performed on a MALDI Biotyper (Bruker Daltonics) with reference database version 7.0.0.0. It queried a reference bank of 7311 spectra. The results were shown as the top 10 matches along with confidence scores ranging from 0.0 to 3.0. The manufacturer recommends a score between 2.0 and 3.0 for high-confidence species identifications, between 1.7 and 1.99 for low-confidence identifications, and less than 1.70 for nonreliable identifications. Therefore, with a score of < 2.0, the phenotypic identification was confirmed by molecular 16S rRNA gene bacterial sequencing using the universal primers 8UA (5′-AGAGTT TGATCMTGGCTCAG-3′) and 1485B (5′-ACGGGCGGT GTGTRC-3′) described by Masaki et al [14]. A sequence similarity $\geq$ 99% with those of the GenBank BLAST and BiBi phylogenetic tools allowed us to identify the bacterium.

Antimicrobial susceptibility testing was performed using a broth microdilution assay with dried microplates (Eiken Chemical Co, Ltd), which is recommended by the Clinical and Laboratory Standards Institute (CLSI; M45-A2). Intermediate resistance was included in the definition of resistance. The β-lactamase activity was determined by the nitrocefin-method for isolated clinical pathogens.

Statistical analyses

The Fisher exact test was used to determine the significance of the differences in the clinical findings and organisms among the differential ocular pathologies. Probability values below .05 were considered to be significant. All statistical

analyses were performed using SPSS software version 16.0 (SPSS Japan).

Results

Demographic data

Sixteen eyes of 16 Japanese patients (7 right eyes, 9 left eyes) were treated for ocular infections caused by a *Moraxella* species during this study period. There were 9 men and 7 women, and ten were patients at Gifu University Graduate School of Medicine, and six, at Ogaki Municipal Hospital. The median age at presentation was 72.0 years, with a range of 3 months to 81 years (mean, 59.1 ± 27.5 years).

Systemic features

A predisposing systemic condition was identified in 15 patients: diabetes in 3 patients, and hypertension, heart disease, lung disease (asthma and pulmonary aspergillosis), malignant disease, and herpes infection (gingivostomatitis and shingles on the face) in 2 patients for each. One patient had a history of myelodysplastic syndrome; one, of atopic dermatitis; and one, of dementia. None of the patients were alcoholic or malnourished, and none was using steroid drops at the time of presentation.

Bacteriologic profile

Sixteen isolates were identified, as follows: *M catarrhalis* in 6 eyes, *M lacunata* in 6 eyes, *M nonliquefaciens* in 3 eyes, and *M osloensis* in 1 eye. The respective scores ranged from 2.055 to 2.623, 1.75 to 1.942, 2.181 to 2.392, and 1.942. All the scores of *M catarrhalis* and *M nonliquefaciens* were given a value of > 2.0. The *M catarrhalis* identification using ID test NH-20 Rapid also yielded a definitive identification. The sequence of the 16S rRNA gene showed a high interstrain similarity (> 99%) for each *M lacunata* strain (accession numbers AF005162.1 and NR_114416.1) and for *M osloensis* (accession numbers AB643590.1 and AB643586.1) [15].

Ocular findings

Nine patients developed keratitis; four, conjunctivitis; and three, blebitis.

Keratitis (Table 1)

The average age of the 7 male and 2 female patients was 54.3 ± 27.2 years, with a range from 6 to 81 years. The right eye was affected in 4 patients, and the left eye, in 5 patients. The presenting complaints were eye pain in 6 eyes, foreign-body sensation in 3 eyes, epiphora and blurred vision in 2 eyes, and hyperemia in 1 eye. Five patients had the most common ocular conditions, ie, eye trauma in 2 patients, soft contact lens wear in 1 patient, use of antiglaucoma eye drops in 1 patient, and a blind eye in 1 patient. *M lacunata* was found in 6 eyes, and *M catarrhalis*, *M nonliquefaciens*, and *M osloensis,* in 1 eye each. *M lacunata* was found only in patients with keratitis (Fisher exact test; $P = .0114$). *Moraxella* was isolated from corneal scraping in all the patients.

For the classification [5], a ring abscess was found in the central cornea in 6 patients, an irregular amoeba-shaped infiltrate overlying the entire cornea was observed in 2 patients, and small round infiltrates were seen in 1 patient (Fig. 1). Eight patients had a hypopyon. The polymicrobes *Staphylococcus hominis* (n = 1), *S capitis* (n = 1), *Acinetobacter* (n = 1), *Corynebacterium* species (n = 1), and *Dermacococcus* species (n = 1) were detected along with the *Moraxella* species in 3 eyes and considered to be normal bacterial flora.

All the patients were treated as inpatients during the intensive medical treatments. Most were treated with antibacterial agents applied by a combination of eye drops, subconjunctival injections, and systemic applications. Topical cefmenoxime was used in all the cases. Topical fluoroquinolone (levofloxacin or moxifloxacin) was used in 8 patients, and tobramycin combined with cefmenoxime, in 1 patient. A subconjunctival injection of ceftazidime and/or vancomycin was used once a day for several days in 7 patients. Systemic antibiotics were used in 8 patients. Five patients used steroid drops to control the inflammation caused by the infection. To treat persistent epithelial defects, 2 patients were given autologous serum eye drops and 1 patient wore a therapeutic soft contact lens. The mean time for complete closure of the epithelial defect in the eyes with keratitis was 24.3 ± 11.4 days (range, 12–42 days).

The BCVA at presentation was > 1.0 logMAR units in 7 of the 9 eyes with keratitis. The final BCVA was ≤ 1.0 logMAR units in those 7 eyes. None of the patients developed a corneal perforation. The visual outcomes after treatment were favorable for all except 1 patient who had to undergo enucleation because of glaucoma and difficulty in determining an appropriate antibacterial therapy.

Conjunctivitis

Moraxella species were isolated from 4 eyes of 4 patients with conjunctivitis (2 right, 2 left eyes). All the patients were women, and each complained of ocular discharge. Their median age was 74.5 years, with a range of 3 months to 81 years (mean, 57.1 ± 37.9 years). The presenting complaints were eye discharge and hyperemia in 3 eyes, blurred vision in 2 eyes, and epiphora and eye pain in 1 eye. Three of the

Table 1 Characteristics of patients with *Moraxella* keratitis

Age, y	Sex	R/L	DM	Background	Ocular predisposing factor	Type	*Moraxella* species	BCVA, logMAR		Treatment						Epithelial defect healing, d
										Antimicrobial therapy			Steroid eye drop	Serum eye drop	SCL wear	
								Initial	Final	Instillation	Subconjunctival	Systemic				
6	F	L	–	–	Trauma	Ring-shaped	*M lacunata*	0.4	− 0.1	CMX+TOB	–	CZOP	+	–	–	17
59	M	R	–	Hypertension	Unknown	Ring-shaped	*M lacunata*	2.0	0.4	LVFX+CMX	VCM+CAZ	CZOP	None	–	–	17
56	M	L	–	OAG	Antiglaucoma eye drops	Ring-shaped	*M lacunata*	2.0	0.2	LVFX+CMX	VCM+CAZ	IPM/CS	+	+	–	25
72	F	L	+	Heart disease, SOAG	Unknown	Ring-shaped	*M non-liquefaciens*	NLP	Prosthesis	LVFX+CMX	VCM+CAZ	–	None	–	–	–
80	M	R	–	Dementia, lung disease	Unknown	Irregular	*M lacunata*	2.0	1.5	LVFX+CMX	VCM+CAZ	IPM/CS MEPM CTRX	+	+	+	42
20	M	R	–	Atopic dermatitis	Contact lens wear	Small round	*M osloensis*	− 0.1	− 0.1	LVFX+CMX	–	MINO	+	–	–	14
39	M	R	–	–	Trauma	Ring-shaped	*M lacunata*	0.5	− 0.2	LVFX+CMX	VCM+CAZ	CTRX	+	–	–	12
81	M	L	–	Asthma	Unknown	Irregular	*M catarrhalis*	CF	2.0	MFLX+CMX	VCM+CAZ	CTRX	None	–	–	39
76	M	L	–	MDS	Unknown	Ring-shaped	*M lacunata*	2.0	0.3	LVFX+CMX	CAZ	CTRX	None	–	–	28

DM diabetes mellitus, *OAG* open-angle glaucoma, *SOAG* secondary open-angle glaucoma, *MDS* myelodysplastic syndrome, *SCL* soft contact lens, *BCVA* best-corrected visual acuity, *NLP* no light perception, *CF* counting fingers, *CMX* cefmenoxime, *TOB* tobramycin, *LVFX* levofloxacin, *MFLX* moxifloxacin, *VCM* vancomycin, *CAZ* ceftazidime, *CZOP* cefozopran, *IPM/CS* imipenem/cilastatin, *MEPM* meropenem, *CTRX* ceftriaxone, *MINO* minocycline

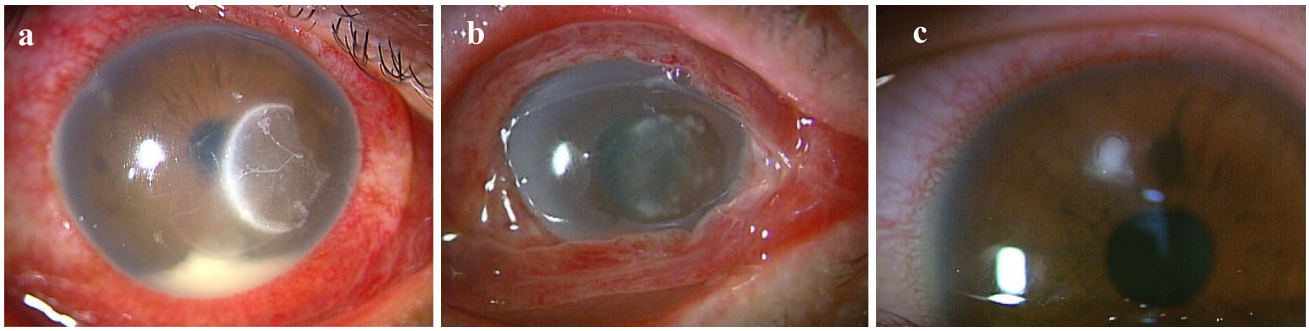

Fig. 1 Slit-lamp photographs of *Moraxella* keratitis. **a** Photograph showing a ring abscess with a hypopyon (59-year-old man). **b** Photograph showing an irregular infiltrate (80-year-old man). **c** Photograph showing a small round infiltrate (20-year-old man). (See Table 1)

four were patients of our hospital. *Moraxella* was isolated from conjunctival swabs in all 4 patients; *M catarrhalis* was found in 3 eyes, and *M nonliquefaciens,* in 1 eye. None of the eyes had polymicrobial isolates. All of the 4 patients were treated with single antibacterial eye drops: levofloxacin in 3 patients and cefmenoxime in 1 patient.

The initial BCVA was 0.1 logMAR units in 2 patients and 0 logMAR units in 1 patient. The final BCVA remained unchanged. The visual acuity could not be examined in the other 2 patients because one was in poor condition and the other was accompanied by a young baby.

Blebitis, a bleb-related infection

Moraxella species were isolated from 1 right eye and 2 left eyes of 3 patients. In all 3 eyes, the stage of the bleb-related infection was blebitis. These patients comprised 2 men and 1 woman; their respective ages were 72, 78, and 79 years. The presenting complaints were hyperemia in 2 eyes, pain in 2 eyes, and discharge in 1 eye. The infection developed after trabeculectomy with adjunctive antimetabolites (mitomycin C in 2 eyes, 5-fluorouracil in 1 eye) in all the cases. The respective onsets of blebitis were 0.8 years, 22.8 years, and 20.3 years after the filtering surgery. However, 1 eye was receiving additional medical treatments after the surgery, and all eyes had maintained the intraocular pressure below 20 mmHg. One patient each was being treated topically for glaucoma, blepharitis, and bullous keratopathy.

M catarrhalis was found in 2 eyes, and *M nonliquefaciens*, in 1 eye. *Moraxella* was isolated from the bleb surface in all these patients. Polymicrobes (*Propionibacterium acnes* [n = 2] and *Corynebacterium* species [n = 1]) were detected along with the *Moraxella* species in all 3 eyes and considered to be normal bacterial flora. All the patients were followed with topical antibacterial therapy. Topical levofloxacin was combined with cefmenoxime in the 2 patients who were not treated with systemic antibiotics. Topical erythromycin was used in 1 patient who received a single subconjunctival injection of ceftazidime and/or vancomycin combined with oral minocycline. The time taken for complete improvement in the blebitis ranged from 10 to 26 days.

The initial BCVA was 0.05 logMAR units in 1 of the 3 eyes. The BCVA of the 1 eye with bullous keratopathy was no light perception and that of the other was hand motions before the infection. The final visual acuity remained unchanged in all eyes.

Antimicrobial susceptibility and β-lactamase activity

The minimum inhibitory concentrations (MICs, μg/mL) for each *Moraxella* species are shown in Table 2. Antimicrobial susceptibility determinations were made for only a few antibiotics (Table 2). All the isolates were sensitive to levofloxacin, tobramycin, ceftazidime, ceftriaxone, chloramphenicol, and minocycline. The respective sulfamethoxazole/trimethoprim susceptibility rates for *M lacunata*, *M catarrhalis*, *M nonliquefaciens*, and *M osloensis* were 100%, 67%, 33%, and 0%. All of the *Moraxella* species were resistant to clindamycin and vancomycin.

Fifteen of the 16 isolates were positive by the nitrocefin disk method. *M osloensis* was negative only for β-lactamase production.

Discussion

Moraxella species are rare causative pathogens of keratitis, with a reported rate of 3.0 to 3.9% of all isolated bacterial keratitis cases [16, 17]. Tan et al [18] reported a significantly increasing trend of *Moraxella* keratitis over a 12-year period. Despite aggressive medical and surgical therapy, the visual prognosis is still generally poor [19].

Several *Moraxella* species are known to infect the human eye including *M catarrhalis*, *M nonliquefaciens*, *M lacunata*, *M osloensis*, *M atlantae*, and *M liquifaciens*. Among these, *M lacunata* is a common cause of corneal ulcers [20]. *M lacunata* is a gram-negative rod of low virulence that is

Table 2 Antimicrobial susceptibility in *Moraxella* species

Antimicrobial agent	*M lacunata* (n = 6)		*M catarrhalis* (n = 6)		*M nonliquefaciens* (n = 3)		*M osloensis* (n = 1)	
Penicillin G	0.25–1		4– >4		0.25–2		0.5	
Ampicillin	≦ 0.12–1		1– >4		≦ 0.12–0.5		≦ 0.12	
Ceftazidime	≦ 0.06–0.12	S = 6	≦ 0.06–0.25	S = 6	≦ 0.06–0.25	S = 3	≦ 0.06	S
Ceftriaxone	≦ 0.06–0.5	S = 6	≦ 0.06–1	S = 6	0.12–0.25	S = 3	≦ 0.06	S
Cefazolin	≦ 0.06–0.5		≦ 0.06– >2		≦ 0.06–2		0.25	
Levofloxacin	≦ 0.12	S = 6	≦ 0.12–0.5	S = 6	≦ 0.12	S = 3	0.25	S
Chloramphenicol	0.5–1	S = 6	≦ 0.25–1	S = 6	0.5–1	S = 3	1	S
Sulfamethoxazole/trimethoprim	10	S = 6	≦ 5–20	S = 4, I = 2	10–20	S = 1, R = 2	20	R
Clindamycin	> 0.5	R = 6	> 0.5	R = 6	> 0.5	R = 3	> 0.5	R
Erythromycin	0.12–0.5		≦ 0.06–0.5		0.5– >16		≦ 0.06	
Tobramycin	0.5–2		1–2		1		1	
Imipenem/cilastatin	≦ 0.12		≦ 0.12		≦ 0.12		≦ 0.12	
Meropenem	≦ 0.015		≦ 0.015		≦ 0.015		≦ 0.015	
Minocycline	≦ 0.12		≦ 0.12–0.5		≦ 0.12		≦ 0.12	
Vancomycin	> 1		>1		>1		> 1	

n number of eyes, *S* susceptible, *I* intermediate, *R* resistant

most commonly associated with subacute conjunctivitis and angular blepharoconjunctivitis and occasionally with upper respiratory tract infections [21, 22]. *M nonliquefaciens* is an important cause of keratitis [5, 23]. *M osloensis* has been isolated from healthy human respiratory tracts and reported to be a rare pathogen in immunocompromised individuals [24]. In the eye, *M osloensis* has been isolated from eyes with keratitis and delayed-onset bleb-associated endophthalmitis [3, 6]. *M lacunata* was the most commonly isolated organism in eyes with keratitis in our cohort (6 of 9: 67%). Why *M lacunata* was detected only in keratitis and not in blepharitis or conjunctivitis and why neither *M atlantae* nor *M liquefaciens* was the cause of the infectious keratitis in any of the patients with it was not determined. Inoue et al speculated that the species of *Moraxella* causing keratitis could be dependent on the time period and geographic location, and it was likely that *M lacunata* and *M nonliquefaciens* are causative pathogens of keratitis in Japan [5]. Unfortunately, we could not determine the exact prevalence and clinical features of keratitis caused by each *Moraxella* species because the number of cases in this study was too small.

The presence of irregular or feathery border lesions with hypopyon was associated with fungal keratitis, and some of the *Moraxella* keratitis patients also showed these signs. In a previous report, corneal specialists correctly differentiated bacterial from fungal keratitis by the photographs more often correctly than by chance, but in fewer than 70% of the cases [25]. Certain clinical signs of infectious keratitis may have a bacterial or fungal etiology; however, it is most important that appropriate microbiologic testing be performed during the initial clinical encounter [25].

Moraxella keratitis is often associated with compromised host immunity caused by chronic alcoholism, malnutrition, and diabetes. Poor sanitary habits are also considered to be important predisposing factors [3, 4]. In individuals aged older than 60 years, diabetes mellitus was an important systemic predisposing factor for *Moraxella* infections [5]. Tobimatsu et al [6] reported that local predisposing factors were significantly more frequent than systemic ones. Cobo et al [23] reported that *Moraxella* infections can occur in patients without any history of trauma or in healthy individuals. We found that the features of corneal infection due to *Moraxella* occurred in both young and older patients, and all the patients had chronic epithelial abnormalities before the onset of keratitis (n = 5) or some predisposing systemic conditions (n = 6). Among these, only 1 patient had a history of diabetes. One patient had dementia, which could become more common in a rapidly aging society. In these patients, we should take care to prevent ocular trauma.

Various antibiotics have been recommended for treatment of *Moraxella* keratitis. Garg et al [26] noted a resistance of *Moraxella* species to cefazolin in 45% of the isolates. Tan et al [19] reported that *Moraxella* species were 100% susceptible to chloramphenicol and cefuroxime and 99% susceptible to ciprofloxacin, but only 88% susceptible to ofloxacin. Berrocal et al [1] reported that all isolates were sensitive to ceftazidime, ciprofloxacin, and aminoglycosides, although they were resistant to vancomycin. Inoue et al [5] demonstrated that *Moraxella* isolates had no resistance to levofloxacin and tobramycin. Our results showed that all stored *Moraxella* species that were isolated from the ocular surface could be investigated for the MIC level in vivo at

the same time. All the isolates were sensitive to levofloxacin, tobramycin, ceftazidime, ceftriaxone, chloramphenicol, and minocycline. Some isolates were resistant to cefazolin, and all of them were resistant to vancomycin. Our results showed that isolated *Moraxella* had high susceptibility to antimicrobials other than vancomycin. We initially used a subconjunctival injection of vancomycin and ceftazidime for empiric therapy; however, we did not use vancomycin after identification of a *Moraxella* species.

Because a higher concentration would generally be achieved by focal administration, ie, eye drops or a subconjunctival injection, than would be achieved by intravenous administration, the sensitivity from MIC does not always reflect or predict the clinical outcome. Then, some antibiotic eye drops will have sufficient sensitivity. The specificity of vancomycin for gram-positive bacteria stems from the protection of their peptidoglycan layer. Gram-negative bacteria are surrounded by a thin peptidoglycan cell wall, which itself is surrounded by an outer membrane containing lipopolysaccharide. A high concentration of vancomycin cannot be active against gram-negative bacteria (*Moraxella* species) owing to an inability to penetrate their outer membrane.

Duo therapy, eg, a combination of a fluoroquinolone and cefmenoxime, may be an effective treatment for keratitis. Suzuki et al [27] reported that a combination of a fluoroquinolone and cefmenoxime had synergistic effects and increased the antibacterial activity of each agent against gram-negative rods. Also in this study, duo therapy was done in all the cases of keratitis and was effective. Consequently, most eyes did not require adjunctive surgical procedures, eg, penetrating keratoplasty, but only 1 eye, a blind eye, was enucleated.

The time of *Moraxella* keratitis healing was very slow, and the reason was not determined. Because persistent inflammation of the cornea was suggested to require a long time to resolve [5], we additionally used steroid drops in 5 patients. To treat persistent epithelial defects, some patients were treated with topical autologous serum or therapeutic soft contact lenses. But the mean time taken for the closure of the epithelial defect was 24 days, which is the same as that reported previously [4, 5].

Endophthalmitis caused by *Moraxella* species is mainly bleb-associated delayed-onset endophthalmitis [1, 28]. Cornut et al [9] reported that *Moraxella* species accounted for 6.5% of the bacterial spectrum for endophthalmitis and that they were always associated with bleb-related and delayed-onset cases of endophthalmitis. Previous studies reported a frequency of 4% to 15% *Moraxella* species among patients with delayed-onset filtering bleb-associated endophthalmitis [29, 30].

The treatment of a *Moraxella* species bleb-related infection depended on the stage of infection, and the treatment was followed by antibacterial therapy consisting of combined therapy of eye drops (levofloxacin and cefmenoxime), ointment, (ofloxacin), subconjunctival or intraocular injections of antibiotics (vancomycin and ceftazidime), and systemic antibiotic (cephalosporins or carbapenems) therapy [31]. Therefore, special attention should be directed to bleb-related infection caused by *Moraxella* because the *Moraxella* species can be resistant to vancomycin.

The relatively good visual acuity of bleb-related infection induced by *Moraxella* was confirmed in a previous study [1]. An analysis of the individual organisms of gram-negative cases in bleb-associated endophthalmitis showed that *Pseudomonas* and *Serratia* cases had poorer mean visual acuity outcomes than did *Moraxella* cases [10]. Additionally, the prognosis of bleb-related infections depended on the stage of the infection, and early diagnosis and treatment was crucial for an optimal visual outcome [32]. Filtering bleb-associated delayed-onset endophthalmitis caused the organisms to invade the intraocular media by a transconjunctival route from the superficial ocular infection, so it is called blebitis (early stage). Then in our cases, delayed-onset endophthalmitis related to *Moraxella* was avoided by recognizing and treating the blebitis. The final visual acuity remained unchanged in all 3 cases; however, the results may be limited for comparisons because of the poor baseline visual acuity of most glaucomatous eyes.

In infants and older children, bacterial conjunctivitis is most often caused by *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *M catarrhalis* [7]. Nihonyanagi et al [33] reported that the frequency of conjunctivitis due to *M catarrhalis* from 2009 to 2014 increased consistently with age, especially in children aged up to 5 years and in adults aged older than 65 years. We found *Moraxella* conjunctivitis in both a 3-month-old baby and an adult aged older than 70 years. We isolated *M catarrhalis* and *M nonliquefaciens* in these 4 patients. All the patients consulted our hospital (3 as inpatients, 1 as an outpatient). The diagnosis of most bacterial conjunctivitis cases is made clinically, and the use of topical antibiotics for bacterial conjunctivitis can be associated with modest improvements in the detection rates of clinical and microbiologic omissions. Then, the majority of conjunctivitis may be managed successfully without cultures. However, we collected a conjunctival swab for culture to aid in the diagnosis of patients presenting with or developing a red, sticky eye, eg, pseudogonococcal ophthalmia neonatorum [34]. All the patients were treated with single topical antibacterial therapy, unlike patients with blebitis or keratitis.

We identified all the *Moraxella* species from ocular specimens by MALDI-TOF MS and biochemical methods. *M lacunata*, *M nonliquefaciens*, and *M osloensis* were identified as causative pathogens of keratitis, and *M nonliquefaciens,* as a causative pathogen of conjunctivitis and blebitis. The etiologic agents of corneal ulcer have changed overtime,

and it is important to accurately identify organisms and perform antimicrobial susceptibility tests.

Among the *Moraxella* species, *M catarrhalis*, *M lacunata*, and *M nonliquefaciens* are known to produce β-lactamase, which degrades penicillin and is part of the first-generation cephalosporins. It remains to be determined whether *M osloensis* produces β-lactamase. We found that 15 isolates of *M catarrhalis*, *M lacunata*, and *M nonliquefaciens* were positive for β-lactamase production. The appropriate treatment for *M osloensis*, a *Moraxella* species not producing β-lactamase, has not been conclusively determined [35]. We have reported that it was susceptible to penicillins (MIC; penicillin G = 0.5, ampicillin = ≤ 0.12; Table 2), cephalosporins, and minocycline. However, penicillin-resistant strains of *M osloensis* have been reported [36]. We chose combined therapy of topical levofloxacin and cefmenoxime and systemic minocycline as a therapy regimen similar for their effectiveness against other *Moraxella* species. The time taken for complete closure of the epithelial defect was 14 days, which is shorter than that of other *Moraxella* species.

In conclusion, ocular infections caused by *Moraxella* species occur in both young and old patients with systemic comorbidities. Four species of *Moraxella* were identified by mass spectrometry, and *M lacunata* was the most frequently isolated organism for keratitis. The use of monotherapy, such as topical fluoroquinolone, remains a good first-line antibiotic treatment against conjunctivitis; however, duo-therapy with a combination of fluoroquinolone and cefmenoxime should be considered for patients who do not respond to monotherapy, such as those with keratitis and bleb-associated infections.

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