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Infantile Acute Conjunctivitis Induced by β-Lactamase–Positive Amoxicillin–Clavulanate–Resistant Strain of *Haemophilus influenzae*: A Report of Three Cases

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Abstract: This study is a retrospectively recruited case series. We report three infants with acute conjunctivitis induced by β-lactamase–positive, ampicillin/clavulanic acid-resistant strains of *Haemophilus influenzae* (BLPACR). Patients with BLPACR-positive cultures were recruited from among 5,107 patients with inflammatory diseases of the ocular surface who underwent examinations, including bacterial culturing of conjunctival sac or corneal scrapings, between 2000 and 2015. Three BLPACR-positive patients were recruited, including a 10-month-old boy, a 4-month-old girl, and a 7-month-old girl. All three demonstrated BLPACR conjunctivitis. The clinical findings in these patients included fever, mucopurulent discharge, lid swelling, and conjunctival hyperemia. Samples of conjunctival swabs were obtained from all three infants, and BLPACR was isolated from all these conjunctival swabs. Antimicrobial susceptibility testing showed sensitivity to levofloxacin and resistance to ampicillin, cefaclor, and clarithromycin. We conclude that in infantile BLPACR conjunctivitis, simultaneous investigation for the determination of causative organism and antibiotic susceptibility testing are crucial aspects of the medical treatment.

Key Words: β-lactamase–positive—Ampicillin/clavulanic acid-resistant strain—*Haemophilus influenzae*—Conjunctivitis—Infant—Levofloxacin.

(*Eye & Contact Lens* 2019;45: e11–e14)

Bacterial-induced acute conjunctivitis is a common ocular disorder in children.[1] The types of bacteria that frequently cause acute bacterial conjunctivitis in children include *Haemophilus influenzae*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Mollaxela catarrhalis*.[1,2] *Haemophilus influenzae*–induced conjunctivitis is the most common type of acute conjunctivitis, manifesting in infants as conjunctivitis-otitis syndrome.[2]

Haemophilus influenzae is a gram-negative rod bacterium and one of the critical causative organisms of respiratory tract infections, acute otitis media, pneumonia, and purulent meningitis. Various types of drug-resistant *H. influenzae* have been isolated from infected patients. Ampicillin-resistance mechanisms in *H. influenzae* include enhancement of TEM-1 or ROB-1 β-lactamase production and mutations in the *ftsI* gene encoding penicillin-binding protein 3.[3] In clinical diagnosis, drug-resistant *H. influenzae* can be classified into four groups based on differences in antibiotic susceptibility to ampicillin and amoxicillin–clavulanic acid: β-lactamase–negative (nonproducing) strains, ampicillin-susceptible (BLNAS) strains, β-lactamase–negative ampicillin-resistant (BLNAR) strains, β-lactamase–positive (producing) ampicillin-resistant (BLPAR) strains, and β-lactamase–positive, ampicillin/clavulanic acid-resistant (BLPACR) strains.

A BLNAR strain of *H. influenzae* has been shown to induce conjunctivitis,[4] and therapeutic agents have been proposed for this condition.[5] However, BLPACR conjunctivitis (i.e., conjunctivitis caused by a BLPACR strain of *H. influenzae*) is a rare condition and has not been adequately reported in the literature. In this report, we describe three cases of BLPACR conjunctivitis in infants, with an emphasis on the clinical course.

CASE REPORTS

This retrospective study was performed in accordance with the Declaration of Helsinki. After approval from the Institutional Review Board of the Nihon University Itabashi Hospital (RK-170411-11), consecutive BLPACR-positive patients in Nihon University Itabashi Hospital were recruited from 5,107 patients with inflammatory diseases of the ocular surface who underwent examinations including bacterial culturing of conjunctival sac or corneal scrapings, between 2000 and 2015. BLPACR conjunctivitis was identified in 3 of the patients: a 10-month-old boy, a 4-month-old girl, and a 7-month-old girl.

Sample Collection and Culture

For all three infants, cultures of the affected conjunctival sac were obtained before antibiotic treatment. One conjunctival sample was obtained from the lower fornix of the conjunctival sac for each patient using transport media with rayon swabs (Seedswab 2; Eikenkagaku, Tokyo, Japan). The swabs were plated onto chocolate and blood agar and incubated at 37°C for 48 hrs in an

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N. Inada has received research funds from Santen and Otsuka Pharmaceutical; J. Shoji has received speaker's fees from Santen Pharmaceutical, Senju Pharmaceutical, and Alcon pharma; S. Yamagami has received research funds from HOYA Corporation.

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Accepted August 28, 2018.

DOI: 10.1097/ICL.0000000000000556

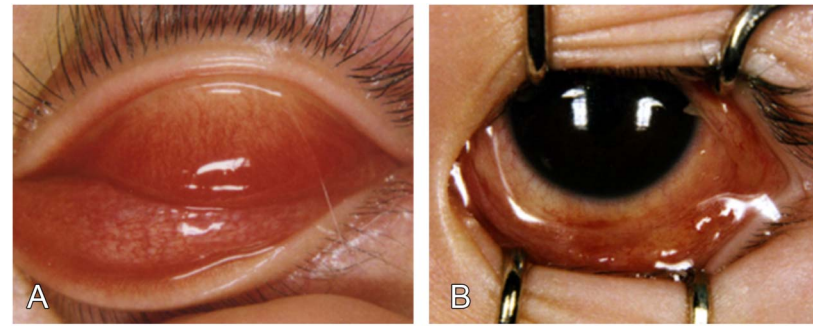

FIG. 1. Palpebral and bulbal conjunctiva photographs for case 1. Severe hyperemia was observed in the palpebral (A) and bulbal (B) conjunctiva, and subconjunctival hemorrhage in the bulbal conjunctiva.

atmosphere containing 5% CO_2. Isolates of *H. influenzae* were identified by colony morphology and conventional methods.

Antibiotic-susceptible and antibiotic-resistant strains of *H. influenzae*, including BLNAS, BLNAR, BLPAR, and BLPACR strains, were identified by antimicrobial susceptibility testing for ampicillin and cefaclor. For antimicrobial susceptibility testing, the production of β-lactamase was assessed using BBL Cefinase paper discs (Nippon Becton Dickinson Company, Tokyo, Japan), and the minimum inhibitory concentration (MIC) was measured using the microdilution method (dry plate "Eiken," Eikenkagaku) in accordance with the Clinical and Laboratory Standards Institute (CLSI) methods. Sensitivity to the following eight drugs was tested: ampicillin, cefaclor, sultamicillin tosilate, cefotaxime sodium, cefditoren pivoxil, imipenem/cilastatin sodium, clarithromycin, and levofloxacin.

The strains of *H. influenzae* were classified according to the CLSI criteria,[6] that is, BLNAS strains, for which the MIC for ampicillin was ≤1 μg/mL; BLNAR strains, with MICs for ampicillin and cefaclor of ≥4 and ≥16 μg/mL; BLPAR strains, with MICs for ampicillin and cefaclor of ≥4 and ≤8 μg/mL; and BLPACR strains, with MICs for ampicillin and cefaclor of ≥4 and ≥16 μg/mL, respectively.

CASE 1

A 10-month-old boy presented with a 1-day history of a discharge, conjunctival hyperemia, and lid swelling in his left eye. He was referred to us for evaluation of an external eye infection. His birth and developmental history were uncomplicated, and his medical history was unremarkable. On ocular examination, we observed conjunctival hyperemia without pseudomembrane, papillary proliferation, follicle formation of the conjunctiva, conjunctival edema, and lid swelling (Fig. 1). Physical examination revealed a temperature of 38.8°C. Computed tomography revealed no evidence of orbital cellulitis or sinusitis (Table 1). A conjunctival swab taken from the left eye before antibiotic treatment yielded *H. influenzae*, which was isolated and characterized as BLPACR by antimicrobial susceptibility testing. We obtained the results of antibiotic susceptibility testing by conjunctival sac culture on day 4. Minor therapeutic effects were obtained after 4 days of instillation of cefmenoxim (4 times daily) and internal use of cefdinir (11 mg/kg/day). However, the boy responded best to treatment with levofloxacin ophthalmic solution (Fig. 2), and the conjunctivitis with lid swelling resolved within 10 days.

CASE 2

A 4-month-old girl presented with a 1-day history of discharge, epiphora, and conjunctival hyperemia in her left eye. She was brought to our clinic for examination of an external eye infection. Her birth and developmental history were uncomplicated. Her medical history included an occurrence of infectious dermatitis of the finger, which was managed with antimicrobial treatment during the 7 days before the occurrence of conjunctivitis. On ocular examination, mucopurulent conjunctivitis was observed in her left eye. Physical examination revealed a temperature of 37.5°C (Table 1). A conjunctival swab taken from the left eye before antibiotic treatment yielded *H. influenzae*, which was isolated and characterized as BLPACR by antimicrobial susceptibility testing (Table 2). We obtained the results of antibiotic susceptibility testing by

TABLE 1. *Clinical Characteristics of Three Infants With BLPACR Conjunctivitis*

	Patient 1	Patient 2	Patient 3
Age	10 months	4 months	7 months
Sex	Boy	Girl	Girl
Ocular findings	Acute conjunctivitis	Acute conjunctivitis	Acute conjunctivitis
Systemic findings	Fever (38.8°C)	Fever (37.5°C)	None
Medical treatment	CMX, LVFX (instillation), and cefdinir (IV)	SBPC (instillation)	SBPC (instillation)
History of antibiotic therapy	No	Yes	Yes
Diagnosis	—	Infectious dermatitis	Meningitis
Causative organism	—	Unknown	BLPAR
Antibiotics	—	CFPN-PI (PO)	CTX and MEPM (IV) CPFX (PO)

BLPAR, β-lactamase–positive ampicillin-resistant strain of *Haemophilus influenzae*; CFPN-PI, cefcapene pivoxil hydrochloride; CPFX, ciprofloxacin hydrochloride; CMX, cefmenoxime; CTX, cefotaxime sodium; IV, intravenous; LVFX, levofloxacin; MEPM, meropenem trihydrate; PO, orally; SBPC, sulbenicillin sodium.

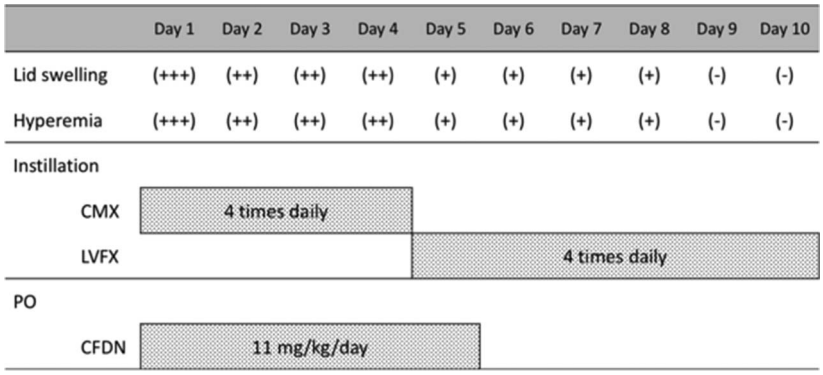

FIG. 2. Clinical approach for case 1. The patient was given antibiotic therapy by instillation of cefmenoxim four times daily for 4 days, and levofloxacin four times daily for another 5 days, in addition to administration of cefdinir at 11 mg/kg/day for 5 days. +, mild; ++, moderate; +++, severe. CMX, cefmenoxime; LVFX, levofloxacin; CFDN, cefdinir; PO, orally.

conjunctival sac culture on day 4, but the antibiotic instillation that we prescribed at initial diagnosis was continued because the conjunctival clinical findings were gradually improving. The patient responded to medical treatment with sulbenicillin ophthalmic solution, and the conjunctivitis resolved within 9 days.

CASE 3

A 7-month-old girl presented with a 1-day history of discharge and conjunctival hyperemia in her left eye. She was hospitalized for 3 months with purulent meningitis caused by a BLPAR strain of *H. influenzae*. Her medical history included the occurrence of purulent meningitis, which was managed with antibiotic treatment, when she was 4 months of age (Table 1). On ocular examination, mucopurulent conjunctivitis was observed in her left eye. A conjunctival swab taken from the left eye before antibiotic treatment yielded *H. influenzae*. Isolated *H. influenzae* from the conjunctiva was characterized as BLPACR by antimicrobial susceptibility testing (Table 2). Additional swabs from her nose yielded BLNAS and BLPACR strains. We obtained the results of antibiotic susceptibility testing by conjunctival sac culture on day 4, but the antibiotic instillation that we prescribed at initial diagnosis was continued because the conjunctival clinical findings were gradually improving. The girl responded to medical treatment with sulbenicillin ophthalmic solution, and the conjunctivitis resolved within 10 days.

RESULTS OF ANTIMICROBIAL SUSCEPTIBILITY TESTING

The results of antimicrobial susceptibility testing are shown in Table 2. The three *H. influenzae* strains isolated from the infants showed low susceptibility to penicillin and cephalosporins, with high resistance to ampicillin, cefaclor, and clarithromycin. The three strains also showed high susceptibility to levofloxacin, and differential sensitivity to cefditoren pivoxil.

DISCUSSION

The three infants described in these case reports were diagnosed with BLPACR conjunctivitis based on their clinical presentations, bacterial cultures isolated from the conjunctival sac, and antimicrobial susceptibility testing. The characteristics of clinical presentations in these infants included young age at onset (all three patients were infants), fever, history of infectious diseases treated with antibiotics, and *H. influenzae* isolated from resident flora in nasopharyngeal tissue or an *H. influenzae* infection developed in an organ other than the eyes. Thus, BLPACR conjunctivitis is acquired in infants, and *H. influenzae* isolated from resident flora or from infected tissues in the ear or upper respiratory tract may be associated with the onset of the condition. Therefore, in cases of severe acute conjunctivitis together with systemic symptoms such as fever, we should consider the possibility of concurrence of conjunctivitis and otitis media or upper respiratory tract inflammation due to the *H. influenzae* infection, and an ophthalmologist should evaluate the presence of otitis media and upper respiratory tract inflammation along with conjunctival swab culturing and antimicrobial susceptibility testing. Furthermore, we should base the systemic antibiotic treatment of these patients on the results of the systemic examination and modify empirical management to sensitive antibiotics based on antimicrobial susceptibility testing.

Haemophilus influenzae is the most prevalent causative organism in acute bacterial conjunctivitis in children and frequently occurs concomitantly with acute otitis media as conjunctivitis-otitis media syndrome. The *H. influenzae* strains prevalent in conjunctivitis-otitis media syndrome can be characterized as BLNAS or BLNAR, and previous studies have reported isolation of the same *H. influenzae* strain from eye discharge and middle ear

TABLE 2. *Results of Antimicrobial Susceptibility Testing*

	ABPC	CCL	SBTPC	CTX	CDTRPI	IPM/CS	CAM	LVFX
Patient 1	> 4	32	≧4/2	1	0.25	0.5	8	≦0.12
Patient 2	> 4	> 32	≧4/2	4	0.5	> 1	8	0.12
Patient 3	> 4	16	≦2/1	≦0.03	≦0.015	1	8	≦0.12

Values represent minimum inhibitory concentration (μg/mL).

ABPC, ampicillin; CAM, clarithromycin; CCL, cefaclor; CDTRPI, cefditoren pivoxil; CTX, cefotaxime sodium; IPM/CS, imipenem/cilastatin sodium; LVFX, levofloxacin; SBTPC, sultamicillin tosilate.

liquid in BLNAR infection.[4,7] A high prevalence of nasal carriage of resistant *H. influenzae* strains has been reported in healthy children in Korea, with strains characterized as follows: 22.8% BLNAR, 6.5% BLPACR, 22.6% BLPAR, and 48.1% BLNAS.[8] Therefore, isolation of resistant strains of *H. influenzae* is believed to be a critical problem representative of growing resistance in bacteria. Although BLNAR strains are the most common among isolated resistant strains of *H. influenza*, the isolation rates of BLPACR in nasopharyngeal swabs from young children have rapidly increased since 2007 in Japan.[9] In this study of three infants with BLPACR conjunctivitis, patient 1 showed resistance to all antibiotics except LVFX; by contrast, SBPC ophthalmic solution was an effective medical treatment for patient 2 and patient 3. Thus, in our patients diagnosed with BLPACR conjunctivitis, susceptibility to antibiotics varied. BLPACR strains, which have two mechanisms of antimicrobial resistance, including β-lactamase production and mutations in the *ftsI* gene, show various patterns of resistance against β-lactams. Therefore, antimicrobial susceptibility testing and genetic analysis of *ftsI* mutations are considered necessary in the clinical examination of BLPACR conjunctivitis in the future.

There are no evidence-based clinical guidelines for empirical management of BLPACR conjunctivitis. Therefore, the results of antimicrobial susceptibility testing for BLPACR strains of *H. influenzae* isolated from these three infants (Table 2) may provide critical data for future empirical management of BLPACR strains. All three *H. influenzae* strains isolated from the infants showed high susceptibility to levofloxacin. Although there were differences in the susceptibilities of individual strains, all the strains showed moderate susceptibility to cefditoren pivoxil. These results showed that BLPACR conjunctivitis treatment with ophthalmic solution may be based on fluoroquinolones, including levofloxacin, and additional treatment with systemic antibiotics, including cefditoren pivoxil, may be recommended. Patient 1 was finally cured using levofloxacin instillation. However, because of the limited sample size in this study, it will be necessary to review the effects of antibiotics in a greater number of patients with BLPACR conjunctivitis in the future. The *H. influenzae* b (Hib) vaccine is effective for meningitis, sepsis, and pneumonia (capsule type), but its efficacy for bronchitis and conjunctivitis (noncapsule type) is questionable. It is important to have or develop a vaccine that is capable of preventing highly antibiotic-resistant *H. influenzae* conjunctivitis, so this should be a topic of future research.

In conclusion, in our case series of infantile BLPACR conjunctivitis characterized by fever, mucopurulent discharge, lid swelling, and severe conjunctival hyperemia (pink eye), simultaneous investigation for the determination of the causative organism and antibiotic susceptibility testing were crucial aspects of the medical treatment.

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