ORIGINAL RESEARCH

Involvement of Haemophilus influenzae in Pediatric Cases of Conjunctivitis, Otitis Media, and Bacterial Sinusitis

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INTRODUCTION

Common etiologies of acute otitis media and acute sinusitis include *Streptococcus pneumo- niae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*.[1-5] They are also predominant pathogens of bacterial conjunctivitis in children.[6]

The relationship between purulent conjunctivitis and acute otitis media was first noticed in 1965.[1] Another study conducted by Bodor found that 73% of the 132 children with purulent conjunctivitis had acute otitis media simultaneously.[7] Conjunctivitis–otitis media syndrome, also called conjunctivitis–otitis syndrome, was then denominated in 1982. The incidence of conjunctivitis–otitis media syndrome ranged from 32 to 73% in children with bacterial conjunctivitis.[6,7] Children younger than 2 years were the vulnerable population to this syndrome, and *H. influenzae* was the most correlative pathogen.[1,7-9] After launching of pneumococcal and *H. influenzae* type b vaccines, studies suggest that nasopharyngeal colonization proportion of non-typeable *H. influenzae* (NTHi) is increasing.[10] The global burden of non-invasive infections, including otitis media, acute bacterial paranasal sinusitis, conjunctivitis, and pneumonia, owing to NTHi is also high currently.[11] Antimicrobial susceptibility of isolated bacteria has regularly been recorded in the Clinical Microbiology Laboratory of National Taiwan University Hospital (NTUH) comprehensively. Antimicro- bial resistance rate of *H. influenzae* has increased dramatically in recent years.

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.[12] 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,[13] and therapeutic agents have been proposed for this condition.[14] 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.

MATERIALS & METHODS

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 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.

RESULTS

A total of 99 children was recruited with ages ranging from 5 months to 10 years. The mean age was 30.4 (± 23.3) months with a median of 26 months (IQR 10–45). 52 (68%) patients were ≤ 3 years old. Demographic data and bacterial culture results are described in Table 1. The mean age of the children in which a particular pathogen was identified varied significantly (p = 0.01). Children with *H. influenzae* infection were the youngest, while the mean age of children with *S. aureus* infection was the oldest. The male-to-female ratio was 1.6. Fifty-seven children had conjunctivitis–otitis media syndrome. Six children had unilateral conjunctivitis and 62 children had bilateral conjunctivae involvement. On the other hand, 21 children had unilateral otitis media and 29 had bilateral infection. Affected eyes of one child and ear lesion site of one child were not recorded. Eight (14%) children with conjunctivitis–otitis media syndrome also had acute bacterial paranasal sinusitis at the same time. Ten (14.9%) children, including six boys and four girls, had a diagnosis of conjunctivitis accompanied with acute bacterial paranasal sinusitis. All of them had bilateral ocular lesions.

Table 1: Bacteriological culture results of conjunctiva discharge in children diagnosed of conjunctivitis with acute otitis media and/or acute bacterial paranasal sinusitis.

Gender and age	*H. influenzae* (N = 62)	*M. catarrhalis* (N = 21)	*S. pneumoniae* (N = 7)	*S. aureus* (N = 9)
%	62.6%	21.2%	7%	9%
Mean age ± SD*	27.5 ± 19.6	28.5 ± 19.5	44 ± 25.1	56.6 ± 39
Age group, n				
< 1y	16	5	2	0
1–2y	14	3	0	2
2–3y	12	3	0	3
3–4y	7	5	0	0
4–5y	7	2	3	0
> 5y	5	2	2	4
Male, n (%)	35 (56.4%)	18 (85.7%)	5 (71.4%)	6 (66.6%)

Conjunctivitis accompanied with acute otitis media and/or acute bacterial paranasal sinusitis is most prevalent in March and April, corresponding to spring season in Taiwan. Clusters in household were observed in 35 (51.3%) patients. At least one family member of these children had conjunctivitis and respiratory tract infection with or without fever within one week of illness of index patient. Three families had two children with a diagnosis of conjunctivitis plus acute otitis media and/or acute bacterial paranasal sinusitis contemporary. 8 (11%) children had underlying diseases including biliary atresia, congenital heart disease, Down syn- drome, bronchial asthma, prematurity, and cleft palate. Only 5 (7%) patients needed hospitalization. A 2-year- old boy was admitted for one week due to a complication of preorbital cellulitis. The other three children were hospitalized for contemporary bronchopneumonia or dehydration due to poor oral intake. In addition, a 10-year- old boy had ophthalmological complications of keratitis and corneal ulcer during follow-up. Oral cefixime was administrated initially and the conjunctiva discharge culture yielded methicillin-susceptible *S. aureus*.

One child had recurrent conjunctivitis–otitis media syndrome one and a half years later. Another child had recurrent acute otitis media with complication of complex febrile convulsion required hospitalization two weeks after the initial episode.

Table 2: Antimicrobial susceptibilities of *Haemophilus influenzae*

Agent	No. of isolates tested	No. (%) of indicated susceptibility	
		Susceptible	Resistant
Ampicillin	56	12 (21.4)	44 (78.5)
Amoxicillin-clavulanate	57	46 (80.7)	11 (19.2)
Chloramphenicol	56	51 (91)	5 (8.9)
Cefixime	56	53 (94.6)	3 (5.3)
Cefpodoxime	52	49 (94.2)	3 (5.7)
Cefotaxime	55	55 (100)	0 (0)
Cefuroxime	56	49 (87.5)	7 (12.5)
SXT	56	18 (32.1)	38 (67.8)

DISCUSSION

To our knowledge, our study is the first report demonstrating the link among bacterial conjunctivitis, acute otitis media and acute bacterial paranasal sinusitis. Eight (14%) children with conjunctivitis–otitis media syndrome also had acute bacterial paranasal sinusitis. Ten (14.9%) children had purulent conjunctivitis and acute bacterial paranasal sinusitis without acute otitis media. Nine (90%) of the conjunctival discharge culture yielded *H. influenzae*. In summary, a quarter of the participants had conjunctivitis accompanied with sinusitis with or without acute otitis media in our study. Besides, the participants recovered from acute conjunctivitis when receiving antibiotic treatment for acute otitis media and/or paranasal sinusitis. These findings suggest that there may be a "conjunctivitis–otitis media–sinusitis syndrome" that shares a same pathogenic process as conjunctivitis–otitis media syndrome. Howie and Schwartz et al. had proven high correlations of nasopharyngeal cultures with cultures of middle ear effusion.[15,16] It is plausible that a single microbiological entity may feature in these conditions due to the continuity of the mucosa in the upper respiratory tract and indeed the co-occurrence of conjunctivitis–otitis media syndrome.[17] Because H. influenzae may enter conjunctival sac, middle ear, and paranasal sinus at the same time, our data shows that there is quite a tight association between conjunctivitis and otitis media, and between conjunctivitis and sinusitis. However, the chance of involvement of all the three infection sites concurrently is low. This is the reason why the number of children with all the three infections are low in present study. Although the pathogenesis remains to be elucidated, we postulated that rhinitis caused by viral infection or allergic inflam-mation of the upper respiratory tract may lead to edema of the ostia of Eustachian tubes, nasolacrimal ducts and paranasal sinuses, resulting in obstruction and bacterial superinfection. Eventually, conjunctivitis, otitis media and sinusitis may develop simultaneously. One study on ferret did show that otitis media and sinusitis may occur after challenge of influenza virus followed by *S. pneumoniae*.[18] Because *H. influenzae* is a common inhabit- ant of nasopharynx,[19] it is reasonable for it to become a predominant pathogen for all the three infections. Such an association has been partly mentioned once in one previous report from Taiwan showing that *H. influenzae* prevailed in children with concurrent acute otitis media and acute bacterial paranasal sinusitis.[20]

One thing unusual is that *S. pneumoniae* is the most prevalent pathogen for isolated acute otitis media and isolated acute bacterial paranasal sinusitis, followed by *H. influenzae*.[21] Our study and previous studies all demonstrate that *H. influenzae* exceeds *S. pneumoniae* to become the most prevalent pathogen when otitis media and/or sinusitis are associated with conjunctivitis. Universal 13-valent pneumococcal conjugate vaccination was started in 2013 in Taiwan. Although the use of pneumococcal conjugate vaccine may decrease the pro- portion of infections caused by *S. pneumoniae*, a predominant role of *H. influenzae* has been noted before the pneumococcal conjugate vaccine era. A recent study in Taiwan showed that *S. pneumoniae* remains the most common etiology of acute otitis media.

In contrast to otitis media and sinusitis, *H. influenzae* is the most common etiology of bacterial conjunctivitis. On the other hand, most *S. pneumoniae* strains causing bacterial conjunctivitis lack capsules and are not typeable.[22,23] These facts imply that when compared with *S. pneumoniae*, *H. influenzae* is in general more virulent to conjunctiva, and is less virulent to middle ear and paranasal sinuses. Although unencapsulated

S. pneumoniae may be more virulent to conjunctiva than encapsulated serotypes, unencapsulated strains have a low ability to cause otitis media and sinusitis. This is the reason why *S. pneumoniae* is not prevalent in "conjunc- tivitis–otitis media–sinusitis syndrome.".

Some studies show that neuraminidase produced by *H. influenzae* and unencapsulated *S. pneumoniae* may disrupt heavily sialylated mucosal surface and degrade surface mucin, facilitating the attachment of bacteria and establishment of an infection. However, the exact virulent factor of "conjunctivitis–otitis media–sinusitis syndrome" remained to be investigated.

Many *H. influenzae* strains may produce biofilm which leads to failure of antibiotic treatment and liability of recurrence. One French study showed that biofilm production is low among *H. influenzae* strains associated with conjunctivitis–otitis media syndrome[26]. The recurrence rate of acute otitis media is 4% (2/57) in our study, which is relatively low

comparing with other studies. Small sample size might be one of the explanations.

Crowded environment including many siblings at home or daycare center might facilitate the transmission of *H. influenzae*.[24] Clusters of purulent conjunctivitis and/or otitis media were observed in 47% of families in one study in 1982. We have a similar finding of household clusters in 34 (51%) patients. Clustering of conjunc- tivitis within families also highlights the possibility of *H. influenzae* as the most possible offending pathogen of associated otitis media and sinusitis.

Prevalence of β-lactamase-producing *H. influenzae* differs widely worldwide. Studies have reported percent- ages of β-lactamase-positive *H. influenzae* between 10 and 25% in most regions, including South Africa, Europe, USA, Canada, Central America, and South America). In some regions (Taiwan, Vietnam, Japan, South Korea), β-lactamase-positive strains account for up to 55% of *H. influenzae* with a high prevalence of β-lactamase- negative ampicillin-resistant strains and β-lactamase-positive-amoxicillin-clavulanate resistant strains.

The present study shows an alarming high ampicillin resistance of 80%, and a high amoxicillin-clavulanate resistant rate of 18% for *H. influenzae*. Except for one missing strain, all amoxicillin–clavulanate resistant isolates were β-lactamase-positive-amoxicillin-clavulanate resistant trains. Combination of β-lactamase production and presence of penicillin-binding protein 3 mutations is thought to be the mechanism of resistance.

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

REFERENCES

1. Coffey, J. D. Jr. Otitis media in the practice of pediatrics. Bacteriological and clinical observations. Pediatrics 38, 25–32 (1966).
2. Dickson, G. Acute otitis media. Prim. Care 41, 11–18. https://doi.org/10.1016/j.pop.2013.10.002 (2014).
3. Piglansky, L. et al. Bacteriologic and clinical efficacy of high dose amoxicillin for therapy of acute otitis media in children. Pediatr. Infect. Dis. J. 22, 405–413. https://doi.org/10.1097/01.inf.0000065688.21336.fa (2003).
4. Wald, E. R. et al. Acute maxillary sinusitis in children. New Engl. J. Med. 304, 749–754. https://doi.org/10.1056/nejm19810326304 1302 (1981).
5. Chi, H. et al. Acute otitis media caused by Streptococcus pneumoniae serotype 19A ST320 clone: epidemiological and clinical characteristics. J. Microbiol. Immunol. Infect. 51, 337–343. https://doi.org/10.1016/j.jmii.2016.08.002 (2018).
6. Buznach, N., Dagan, R. & Greenberg, D. Clinical and bacterial characteristics of acute bacterial conjunctivitis in children in the antibiotic resistance era. Pediatr. Infect. Dis. J. 24, 823–828. https://doi.org/10.1097/01.inf.0000178066.24569.98 (2005).
7. Bodor, F. F. Conjunctivitis−otitis syndrome. Pediatrics 69, 695–698 (1982).
8. Bingen, E., Cohen, R., Jourenkova, N. & Gehanno, P. Epidemiologic study of conjunctivitis−otitis syndrome. Pediatr. Infect. Dis.
9. J. 24, 731–732. https://doi.org/10.1097/01.inf.0000172939.13159.3b (2005).
10. Bodor, F. F., Marchant, C. D., Shurin, P. A. & Barenkamp, S. J. Bacterial etiology of conjunctivitis−otitis media syndrome. Pediatrics 76, 26–28 (1985).
11. Spijkerman, J. et al. Long-term effects of pneumococcal conjugate vaccine on nasopharyngeal carriage of S. pneumoniae, S. aureus, H. influenzae and M. catarrhalis. PLoS ONE 7, e39730.https://doi.org/10.1371/journal.pone.0039730 (2012).
12. Van Eldere, J., Slack, M. P., Ladhani, S. & Cripps, A. W. Non-typeable Haemophilus influenzae, an under-recognised pathogen. Lancet. Infect. Dis 14, 1281–1292. https://doi.org/10.1016/s1473-3099(14)70734-0 (2014).
13. Søndergaard A, Nørskov-Lauritsen N. Contribution of PBP3 Substitutions and TEM-1, TEM-15, and ROB-1 beta-lactamases to cefotaxime resistance in Haemophilus influenzae and Haemophilus parainfluenzae. Microb Drug Resist 2016;22:247–252.
14. Sugita G, Hotomi M, Sugita R, et al. Genetic characteristics of Haemophilus influenzae and Streptococcus pneumoniae isolated from children with conjunctivitis-otitis media syndrome. J Infect Chemother 2014;20:493–497.
15. Haas W, Gearinger LS, Usner DW, et al. Integrated analysis of three bacterial conjunctivitis trials of besifloxacin ophthalmic suspension, 0.6%: Etiology of bacterial conjunctivitis and antibacterial susceptibility profile. Clin Ophthalmol 2011;5:1369–1379.
16. Howie, V. M., Ploussard, J. H. & Lester, R. L. Jr. Otitis media: a clinical and bacteriological correlation. Pediatrics 45, 29–35 (1970).
17. Schwartz, R., Rodriguez, W. J., Mann, R., Khan, W. & Ross, S. The nasopharyngeal culture in acute otitis media. A reappraisal of its usefulness. JAMA 241, 2170–2173 (1979).
18. Lewnard, J. A., Givon-Lavi, N. & Dagan, R. Interaction with nontypeable Haemophilus influenzae Alters progression of Streptococ- cus pneumoniae from colonization to disease in a site-specific manner. J. Infect. Dis. 220, 1367–1376. https://doi.org/10.1093/infdi s/jiz312 (2019).
19. Peltola, V. T., Boyd, K. L., McAuley, J. L., Rehg, J. E. & McCullers, J. A. Bacterial sinusitis and otitis media following influenza virus infection in ferrets. Infect. Immun. 74, 2562–2567. https://doi.org/10.1128/iai.74.5.2562-2567.2006 (2006).
20. Sugita, G. et al. Genetic characteristics of Haemophilus influenzae and Streptococcus pneumoniae isolated from children with conjunctivitis-otitis media syndrome. J. Infect. Chemother. 20, 493–497. https://doi.org/10.1016/j.jiac.2014.04.015 (2014).

21. Cho, Y. C. et al. Epidemiology and antimicrobial susceptibility of non-typeable Haemophilus influenzae in otitis media in Taiwanese children. J. Microbiol. Immunol. Infect. 52, 75–80. https://doi.org/10.1016/j.jmii.2017.08.020 (2019).
22. Mather, M. W. et al. A systematic review and meta-analysis of antimicrobial resistance in paediatric acute otitis media. Int. J. Pediatr. Otorhinolaryngol. 123, 102–109. https://doi.org/10.1016/j.ijporl.2019.04.041 (2019).
23. Haas, W., Hesje, C. K., Sanfilippo, C. M. & Morris, T. W. High proportion of nontypeable Streptococcus pneumoniae isolates among sporadic, nonoutbreak cases of bacterial conjunctivitis. Curr. Eye Res. 36, 1078–1085. https://doi.org/10.3109/02713683.2011.624670 (2011).
24. Norcross, E. W., Tullos, N. A., Taylor, S. D., Sanders, M. E. & Marquart, M. E. Assessment of Streptococcus pneumoniae capsule in conjunctivitis and keratitis in vivo neuraminidase activity increases in nonencapsulated pneumococci following conjunctival infection. Curr. Eye Res. 35, 787–798. https://doi.org/10.3109/02713683.2010.492462 (2010).
25. Istre, G. R., Conner, J. S., Broome, C. V., Hightower, A. & Hopkins, R. S. Risk factors for primary invasive Haemophilus influen- zae disease: increased risk from day care attendance and school-aged household members. J. Pediatr. 106, 190–195. https://doi. org/10.1016/s0022-3476(85)80285-7 (1985).