

Characterization of Serotypes and Molecular Drug Resistance Patterns of *Haemophilus influenzae* in Kunming Children

MEI YUAN^{1#}, MINGBIAO MA^{1#}, HONGCHAO JIANG^{2#}, MAO FAN¹, YING SUN³, BAILING ZHOU¹,
XINGXING FENG¹, JUNYI YANG¹, MIN SU¹ and XIAOLI HE^{4*}

¹Department of Laboratory, Children's Hospital affiliated with Kunming Medical University, Kunming, China

²Science and Education Section, Children's Hospital affiliated with Kunming Medical University, Kunming, China

³Epilepsy Center of Children, Children's Hospital affiliated with Kunming Medical University, Kunming, China

⁴Institute of Pediatrics, Children's Hospital affiliated with Kunming Medical University, Kunming, China

Submitted 11 December 2022, accepted 31 January 2023, published online 4 May 2023

Abstract

The present study aimed to determine the capsular serotype distribution and antimicrobial drug resistance patterns of *Haemophilus influenzae* from children in the Kunming region of China. This information could guide policymakers in clinical treatment. In the present study, *H. influenzae* isolates were tested for their serotypes, antimicrobial susceptibility pattern, and presence of β -lactamases. One-hundred forty-eight *H. influenzae* strains isolated from children 0–2 years old were investigated for capsular types by glass slide agglutination and molecular methods, and biotyped by the biochemical reactions. The drug resistance-encoding genes *TEM-1*, *ROB-1*, and the *ftsI* gene mutations *PBP3-3*, and *PBP3-BLN* were detected with real-time quantitative polymerase chain reaction (qPCR). The prevalence of β -lactamase-

producing strains (60.3%) was significantly higher ($p < 0.05$) than non-enzyme-producing strains. β -Lactamase-producing strains were multidrug resistant to various antibiotics such as ampicillin, tetracycline, sulfamethoxazole/trimethoprim, chloramphenicol, cefuroxime, and cefaclor. Among β -lactamase-producing strains, the detection rates of the *TEM-1*, *PBP3-BLN*, *PBP3-s*, and *ROB-1* were 54.1%, 18.9%, 11.8%, and 6.9%, respectively. The biotyping results show that most *H. influenzae* strains were of type II and III. Non-typeable *H. influenzae* (NTHi) accounted for 89.3% of the strains. NTHi strains were the most prevalent in this region; most belonged to biological types II and III. β -Lactamase-positive ampicillin-resistant (BLPAR) strains were prevalent among *H. influenzae* isolates in this region.

Key words: *emophilus influenzae*, biotyping and capsule typing, resistant phenotype

Introduction

Haemophilus influenzae is a common pathogen of community-acquired infections in children (Fu et al. 2021), which is the leading cause of pneumonia, otitis media, conjunctivitis, meningitis, septicemia, and other diseases (Vallejo et al. 2019). *H. influenzae* is classified in various schemes, of which capsule type and biotype are the most common. Bacterial typing is an essential basis for disease epidemiology and vaccine development. Using various biochemical reactions, such as with the indigo substrate, indole, and ornithine decarboxylase, *H. influenzae* can be divided into eight biotypes, namely I–VIII. According to the properties of capsular polysaccharides, *H. influenzae* can also

be divided into six serotypes (from a to f) and non-typeable *H. influenzae* strains (NTHi) (Wen et al. 2020). The drug resistance, disease characteristics, invasiveness, and infection-vulnerable tissues differ between these bio- and serotypes (Crandall et al. 2019; Fuji et al. 2021). The literature data indicates a relationship between the selected biotypes and the sites of their colonization, as well as their association with specific infectious problems (Brabender et al. 1984; Mojgani et al. 2011; Crandall et al. 2019; Heliodoro et al. 2020; Li et al. 2020; Fuji et al. 2021). NTHi has replaced *H. influenzae* type b (Hib) as the leading cause of invasive infection in children; however, it has less concern for pediatricians (Butler et al. 2018; Heliodoro et al. 2020). Different regions and populations showed mixed *H. influenzae*

Mei Yuan, Mingbiao Ma and Hongchao Jiang contribute equally to this work and are co-first authors; Min Su and Xiaoli He co-correspondent.

* Corresponding authors: M. Yuan, Department of Laboratory, Children's Hospital affiliated with Kunming Medical University, Kunming, China; e-mail: yuanmei944@163.com

X. He, Institute of Pediatrics, Children's Hospital affiliated with Kunming Medical University, Kunming, China; e-mail: yes815079752@163.com

© 2023 Mei Yuan et al.

This work is licensed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 License (<https://creativecommons.org/licenses/by-nc-nd/4.0/>).

typing results. The classification of *H. influenzae* in children in the Kunming region of China is unclear.

In recent years, the incidence of *H. influenzae* infection in children has increased, and profiles of antimicrobial resistance among children in China demonstrated that the proportion of *H. influenzae* in all pathogenic bacteria has risen from one-fourth in 2017 to one-third in 2018. It seriously endangered children's health in China (Fu et al. 2021). With the widespread use of antibiotics, the resistance pattern of *H. influenzae* to commonly used antibiotics has also undergone significant changes. Common resistance patterns include β -lactamase-negative ampicillin-resistant (BLNAR), β -lactamase-positive ampicillin-resistant (BLPAR), β -lactamase-negative ampicillin-sensitive (BLNAS), β -lactamase-producing amoxicillin-clavulanate-resistant (BLPACR), and BL-nonproducing ampicillin-intermediate (BLNAI) (Kim et al. 2007). Resistance mechanisms are also becoming more complex, which also complicates the choice of antibiotics for treatment. The mechanism of drug resistance for *H. influenzae* included the production of β -lactamase and mutations of the *fstI* gene, which encodes a penicillin-binding protein (PBP) of low affinity (Li et al. 2020). The former is mainly mediated by the *TEM-1* and *ROB-1* genes, and the latter mainly expresses the *PBP3-S* and *PBP3-BLN* mutations in the *ftsI* gene (Søndergaard and Nørskov-Lauritsen 2016).

Nevertheless, rare drug resistance has not yet been detected in *H. influenzae* strains in southwestern China. The replacement phenomenon and capsular switching are widespread in *Streptococcus pneumoniae*, another microorganism associated with meningitis and pneumonia. Therefore, it is required to monitor the spreading of *H. influenzae* in the post-vaccine period to know the circulating serotypes in the community. It is also desired to know which serotypes are circulating in the region to study this data compared to other regional data. It would help to analyze the factors causing the serotype variation.

This paper studied the serotypes, common drug resistance patterns, and drug resistance genes of *H. influenzae* strains in Kunming. Besides, the scientific basis for the diagnosis, the prevalence and drug resistance of *H. influenzae* in children, as well as the prevention and treatment and prevention of these bacteria, was provided.

Experimental

Materials and Methods

Patients and clinical isolates. This study was approved by the institutional Review Board, and written informed consent was obtained from each participant.

Ethical approval was obtained from the Zhu Futang Clinics of Pediatrics. The patients were recruited at the Children's Hospital affiliated with Kunming Medical University (Kunming, China), where the publication of data generated from this study was approved. Informed consent was obtained from the participants with the option to withdraw from the study at any time. A total of 800 *H. influenzae* strains isolated from children of 0–15 years, diagnosed with *H. influenzae* infection according to the Zhu Futang Clinics of Pediatrics, were recruited at the Children's Hospital affiliated with Kunming Medical University between 2018–2021. *H. influenzae* isolates were identified by satellite phenomenon and the VITEK² NH card (bioMérieux, France) (Fuji et al. 2021). The ATB identification cards were used to test the drug sensitivity. *H. influenzae* ATCC[®] 49247[™] was used as the reference strain for the quality control.

Capsular serotyping and genotyping. Capsule typing was carried out on 24-h subcultures with the Phad-eact[™] Haemophilus Test 50 kit (TaKaRa Company, Japan) (Collins and Kelly 1983). Strains were reported as having a type b capsule, a non-type-b capsule, or as non-typeable isolate. The classic slide agglutination method was used for serotyping (Crandall et al. 2019). *H. influenzae* bacterial suspension was mixed with specific antiserum of *H. influenzae* type a, b, c, d, e, f. Slide agglutination occurred within 1 minute, which indicated that the corresponding capsular serotype was positive. If neither aggregated, it was judged as an NTHi strain. All the isolated strains were serotyped by amplifying the *bexA* capsular gene with PCR, as described previously. Strains that PCR could not identify were classified as non-typeable (NTHi) (Falla et al. 1994). PCR was also done to identify the corresponding capsular genes of *H. influenzae* of type “a” to type “f” (Falla et al. 1994). The primer sequences of capsular genes in *H. influenzae* are provided in Table I (Fella et al. 1994).

Biotyping. The biotypes of the isolates were determined by the method described by Kilian (Kilian et al. 1979; Kilian 1985). Liquid media prepared in the laboratory were inoculated with 24-h old or fewer subcultures. Test cultures were incubated in the air for 4 h at 35°C. This biotyping system uses the presence of preformed enzymes, and because growth is not required, the media were not supplemented with X and V factors. According to the difference of reaction results in biochemical reaction tubes of indole, ornithine decarboxylase, and indole, *H. influenzae* strains could be divided into eight biotypes: I (+, +, -), II (+, +, -), III (-, +, -), IV (-, +, +), V (+, -, +), VI (-, -, +), VII (+, -, -), VIII (-, -, -).

Antibiotics susceptibility testing and β -lactamase assay. The minimum inhibitory concentrations (MICs) were determined by the microdilution method according to the CLSI guideline (CLSI 2011). Eighteen

Table I
The primers for capsular genes of *Haemophilus influenzae*
(Fella et al. 1994).

Gene	Primer	Product size (bp)
<i>P6</i>	F: ACGATGCTGCAGGCAATGGT R: CATCAGTATTACCTTCTACTAAT	198
<i>Hi (bexA)</i>	F: CGTTTGTATGATGTTGATCCAGAC R: TGTCCATGTCTTCAAAATGATG	343
<i>Hi-a</i>	F: CTACTCATTGCAGCATTTGC R: GAATATGACCTGATCTTCTG	250
<i>Hi-b</i>	F: GCGAAAGTGAACCTCTTATCTCTC R: GCTTACGCTTCTATCTCGGTGAA	480
<i>Hi-c</i>	F: TCTGTGTAGATGATGGTTCA R: CAGAGGCAAGCTATTAGTGA	250
<i>Hi-d</i>	F: TGATGACCGATACAACCTGT R: TCCACTCTTCAAACCATTTCT	150
<i>Hi-e</i>	F: GGTAACGAATGTAGTGGTAG R: GCTTACTGTATAAGTCTAG	1,350
<i>Hi-f</i>	F: GCTACTTATCAAGTCCAAATC R: CGCAATTATGGAAGAAAGCT	450
<i>GAPDH</i>	F: ACAACTTTGGTATCGTGGAAGG R: GCCATCACGCCACAGTTTC	230

Table II
The primers for drug resistance genes of *Haemophilus influenzae*
(Søndergaard and Nørskov-Lauritsen 2016; Li et al. 2020).

Gene	Primer	Product size (bp)
<i>TEM</i>	F: TAAGAGAATTATGCAGTGCTGCC R: TCCATAGTTGCCTGACTCCCC	458
<i>ROB</i>	F: AAGCCCGCATTGGCGTTTAT R: GGGCAATCGTCATGCCTTTG	521
<i>PBP3S</i>	F: GATACTACGTCCTTTAAATTAAG R: GCAGTAAATGCCACATACTTA	551
<i>PBP3BLN</i>	F: TTCAAGTAACCGTGGTGTGAC R: GCAGTAAATGCCACATATTTTC	465

antibiotics were tested, and the results were interpreted according to CLSI standards (Søndergaard and Nørskov-Lauritsen 2016). *H. influenzae* ATCC® 49247™ and ATCC® 49766™ were used as the quality controls. Production of β-lactamase was detected with the cephalosporin thiophene paper (Oxoid Company, UK). When the paper turned red, it was a positive indicator that the bacteria produced β-lactamase (Yamada et al. 2020). The antibiotic resistance-encoding genes were identified using the primers described in Table II in a conventional PCR.

Statistical analysis. We utilized the chi-squared test to compare the differences among groups. The measurement data were expressed as a mean ± standard deviation. A *p*-value of <0.05 was considered statistically significant. All the analyses were carried out within the SPSS v22.0 (IBM, USA).

Results

Clinical characteristics of pediatric patients. This study characterized 800 *H. influenzae* strains, of which most came from respiratory specimens (738, 92.3%) (sputum and bronchoalveolar lavage fluid). The second primary source of strains was wound secretions (31, 3.8%). The remaining 12 strains (1.5%) were from ocular secretions, 11 isolates were from blood (1.4%), and 8 (1.0%) were from the cerebrospinal fluid. *H. influenzae* strains were identified by the presence of the *P6* gene. The *bexA* gene found in all the subtypes for *H. influenzae* was also sought, and the isolates, which showed the absence of the *bexA* gene, were classified as NTHi isolates. Almost 82% (n=652) of *H. influenzae* isolates were recovered from 2–15 years old children’s samples. The remaining 18.5% of ilates (n=148) were isolated from children of 0–2 years and were vulnerable to *H. influenzae* meningitides and pneumonia. Only these isolates were used for typing purposes.

Typing. One hundred forty-eight *H. influenzae* strains isolated from children (0–2 years) were selected for serotyping. It appeared that 132 strains (89.2%) were NTHi and nine strains (6.1%) were *H. influenzae* type b, whereas four strains (2.7%) were *H. influenzae* type e, two strains (1.3%) were *H. influenzae* type f, and one strain (0.7%) was *H. influenzae* type c1. The corresponding capsule-encoding genes were detected accurately (Fig. 1).

The biotyping showed the presence of 64 (43.2%) type II strains, 55 (37.2%) type III strains, 12 (8.1%) type I strains, and eight (5.4%) type IV strains. Type V and VI were detected in four (2.7%) strains, while only one strain was of type VII. Two blood isolates were identified as NTHi and were of biotypes II and IV. The others blood isolates were *H. influenzae* type b and biotype I. Six wound isolates were nontypeable and of type III. All ocular isolates were of biotype II and were non-typeable.

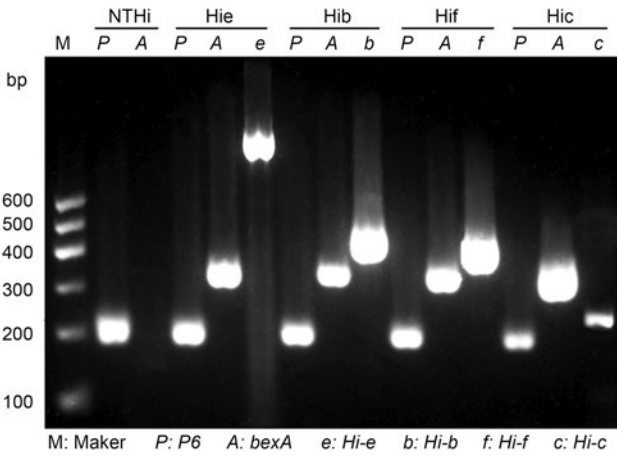


Fig. 1. Electrophoregram of *Haemophilus influenzae* capsular genes’ amplicons, including NT0Hi, Hie, Hib, Hif, and Hic strains.

Table III
Drug resistance rates of *Haemophilus influenzae* strains [n (%)].

Antibiotic	β-lactamase-positive (n = 474)	β-lactamase-negative (n = 326)	χ ²	<i>P</i>
Ampicillin	474 (100.0)	113 (34.6)	45.725	≤0.001***
Sulfamethoxazole/trimethoprim	399 (84.2)	232 (71.2)	14.558	≤0.001***
Cefaclor	266 (56.1)	137 (42.0)	2.515	0.113
Cefuroxime	225 (47.5)	110 (33.8)	0.871	0.351
Amoxicillin/clavulanate	123 (25.9)	109 (33.4)	31.911	≤0.001***
Tetracycline	48 (10.1)	12 (3.7)	3.266	0.071
Chloramphenicol	41 (8.6)	4 (1.2)	10.184	0.001***
Cefotaxime	4 (0.8)	5 (1.5)	1.593	0.207
Ofloxacin	2 (0.4)	1 (0.3)	0.010	0.919
Multidrug resistance	295 (62.2)	106 (32.5)	3.911	0.048*

* – indicates a statistically significant difference; *** – indicates a particularly significant statistical difference

Drug resistance patterns. All the 800 strains were tested for antibiotic resistance using the minimum inhibitory concentrations (MICs) method according to the CLSI guideline (CLSI 2011) using eighteen antibiotics. Among them, 474 (59.5%) strains were β-lactamase positive. In the group of β-lactamase positive strains, 295 were multi-drug resistant (MDR) to numerous antibiotics, such as sulfamethoxazole/trimethoprim, ampicillin, cefaclor, cefuroxime, amoxicillin-clavulanic acid, tetracycline, and chloramphenicol. The rate of MDR in the β-lactamase-positive strains was significantly higher than that of β-lactamase-negative strains (326 (40.5 %)). Among β-lactamase-negative strains, 106 were multi-drug resistant.

Based on the resistance patterns towards ampicillin, amoxicillin, and clavulanic acid, the strains were classified into BLPAR (43.5%), BLNAS (24.6%), BLPACR (16.5%), BLNAR (14.8%), and BLNAI (0.6%) (Table III). The TEM-1-type *bla* gene, PBP3-BLN (*ftsI*) gene, PBP3s gene, and ROB-1-type *bla* gene were detected in 54.1%, 18.9%, 11.8%, and 6.9% of 800 *H. influenzae* strains, respectively. The proportion of BLNAS was 12% of TEM-1-positive strains; 16% of ROB-1-positive strains. The BLNAR detection rate was 60% for PBP3BLN-positive strains, and 40% for PBP3S-positive strains. The BLPACR phenotype was detected in 56% of TEM-1 and PBP3BLN-positive strains. Simultaneously, 28% of β-lactamase-positive strains had both TEM-1 and PBP3s, while 12% of β-lactamase-positive strains had two TEM-1 and PBP3s genes. The PBP3S and PBP3BLN genes were seen together in 4% of the β-lactamase-positive strain (Fig. 2).

Discussion

H. influenzae causes a common community-acquired infection in children and has regional strong characteristics in drug resistance and serotypes (Falla et al.

1994). In Mainland China, limited surveys focused on the epidemiologic data of *H. influenzae* from children, especially those under five years. The surveillance of serotype distribution and prevalence of drug-resistant strains in the general population is critical for the public health governmental departments to develop appropriate prevention protocols for *H. influenzae* infection.

Ampicillin and second-generation cephalosporins are commonly used drugs for treating *H. influenzae* infections. However, they may not be suitable for treatment due to high resistance found in the clinical strains (Heinz 2018; Ubukata et al. 2019). Antibiotics such as third generation cephalosporins and amoxicillin-clavulanate are recommended for children in this area. The results of capsule typing by serological methods and PCR were consistent. The prevalent strains in Yunnan were NTHi, while the capsular *H. influenzae* strains were rare, consistent with the literature (Fally et al. 2021). The current vaccine against *H. influenzae* can only prevent Hib infection. This vaccine is no longer suitable for the current epidemiological situation, where NTHi has become the prevalent type (Dong et al. 2020). Research on new and effective vaccines against NTHi has become the current focus for preventing and treating these bacteria (Bakaletz et al. 2018). According to the difference in the reaction results of *H. influenzae* with the indigo substrate, indole, and ornithine decarboxylase, the bacteria can be divided into eight biotypes, namely I–VIII. The results of this work showed that types II and III were the most prevalent biotypes in children in this area and are different from those reported in the literature. The *H. influenzae* strains from adults in Kunming are mainly of types III and IV, in the neighboring city of Chengdu are mainly of types I and IV, and in Iran are mainly of types I–III (Langereis and de Jonge 2020). Different biotypes cause different manifesting diseases. This study shows that the Hib and biotype I strains are mainly detected in

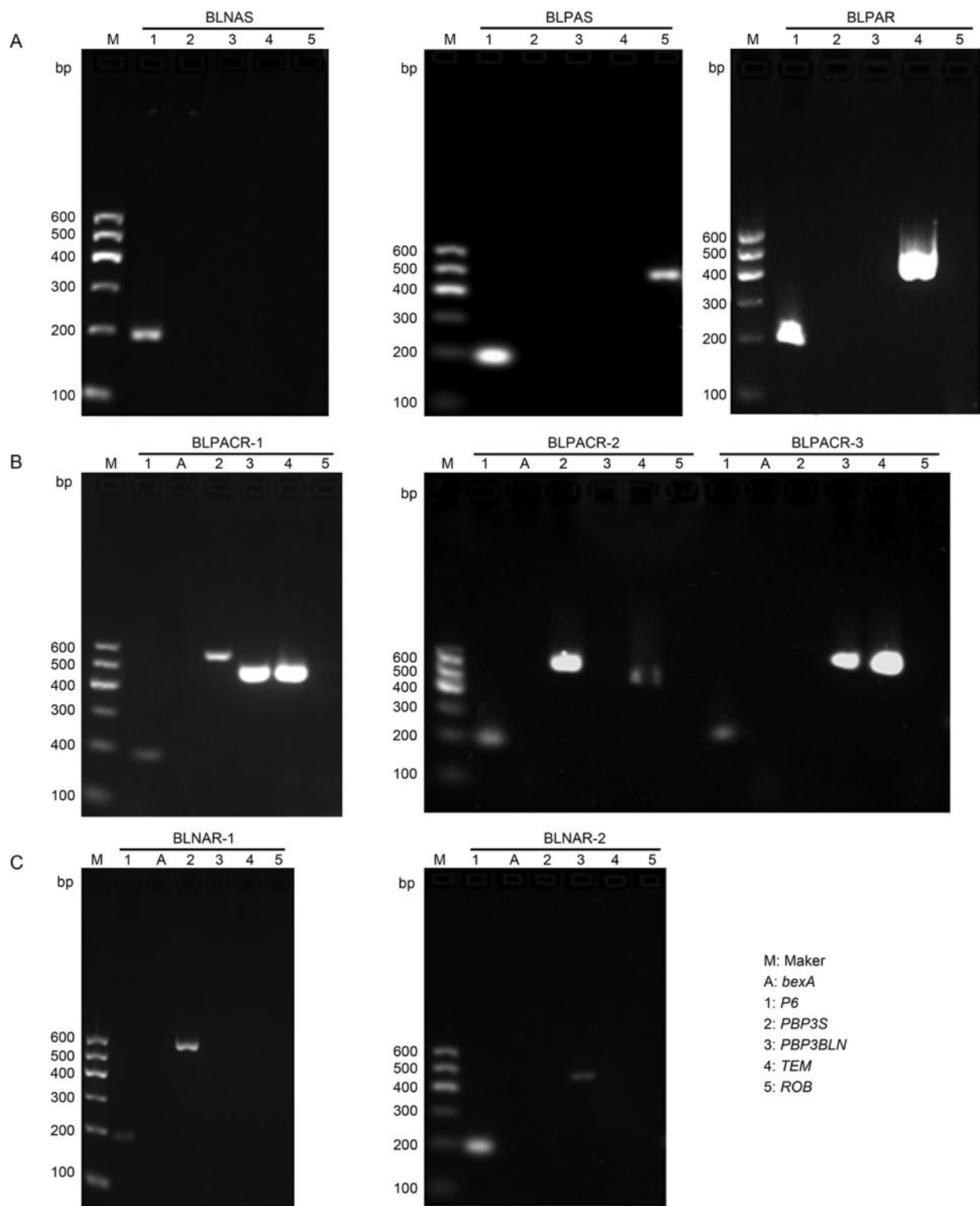


Fig. 2. Electrophoregram of *Haemophilus influenzae* drug-resistance genes (in the BLNAR and BLPACR phenotype strains).

blood and cerebrospinal fluid samples, indicating a specific relationship in their invasion. Type III was mainly detected in wound secretion specimens, which may be associated with cellulitis. Type II was detected in all ocular secretion specimens, which may be related to ocular conjunctivitis. Unlike those reported in the literature, type I often causes acute lower respiratory tract

infection and sepsis in children, type II causes explosive respiratory disease, type III causes tissue cellulitis, and type IV causes neonatal sepsis. However, there are also limits to this study. The above findings' mechanisms were not clarified and still need further exploration.

There were regional differences in the detection rate of β -lactamases, drug-resistance genes, and

drug-resistance patterns. The rate of antibiotic resistance and multidrug resistance of β -lactamase-positive strains was higher than that of β -lactamase-negative strains, indicating that the production of β -lactamase is one of the important reasons for the increased drug resistance and multidrug resistance of *H. influenzae* (Mojgani et al. 2011). In this study, the detection rate of β -lactamase was slightly lower than the national average level of China, as reported in 2022 (Zhou et al. 2022). The detection rate of drug resistance gene *TEM-1* was the highest, indicating that the drug resistance phenotype BLPAR mediated by *TEM-1* β -lactamase-producing strains was the primary drug resistance mechanism in *H. influenzae* (Fu et al. 2021). Mutations in the *ftsI* gene (*PBP3S* and *PBP3BLN*) result in the BLNAR phenotype (Nørskov-Lauritsen et al. 2021). The β -lactamase production and *ftsI* gene mutations work together to result in BLPACR, which is consistent with the resistance mechanism reported in the literature (Schotte et al. 2019). The higher detection rate of BLPAR shows that the most common drug resistance pattern in this region is BLPAR, while BLPACR and BLNAR are also popular, which may be one of the reasons for multidrug resistance (Cherkaoui et al. 2015). This situation requires rigorous attention.

In conclusion, *H. influenzae* is one of the most common pathogens of childhood diseases, invading respiratory tract infections. The common biotypes in this region are II and III, and the common serotype is NTHi. The antibiotic resistance and multi-resistance levels are severe. The resistance rate to ampicillin and second-generation antibiotics is high, so third-generation cephalosporins and amoxicillin-clavulanic acid are recommended for the treatment. The detection rate of drug-resistant phenotypes BLPAR, BLNAR, and BLPACR was high, and BLPAR was the most prevalent. The *TEM-1*, *ROB-1*-type β -lactamase, and *PBP3BLN*, *PBP3S* resistance genes contributed to the observed drug resistance. In short, this region's drug resistance phenotypes and mechanisms are diverse, and the drug resistance situation is complex. It is necessary to monitor the *H. influenzae* drug-resistance phenotypes continuously.

Conclusions

Non-typeable *H. influenzae* strains are an important cause of bacteremic pneumonia and meningitis in children 0–2 years from the Kunming region. The dominant biotypes were II and III. The BLPAR was the most often detected resistant phenotype among NTHi isolates.

Availability of data and material

The data used to support this study are available from the corresponding author upon request.

Funding

This work was supported by Kunming Health and Technology Personnel training project and “Ten hundred thousand” project training, 2021-SW(reserve)-70; Kunming Health and Technology Personnel training project, 2022-11-01-006; Kunming Health and Technology Personnel training project and “Ten hundred thousand” project training 2021-SW(reserve)-109; Kunming Health and Technology Personnel training project and “Ten hundred thousand” project training, 2020-SW-118; Kunming Health and Technology Personnel training project, 2022-SW(technology)-007.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

- Bakaletz LO, Novotny LA. Nontypeable *Haemophilus influenzae* (NTHi). Trends Microbiol. 2018 Aug;26(8):727–728. <https://doi.org/10.1016/j.tim.2018.05.001>
- Brabender W, Hodges GR, Barnes WG. Clinical significance of serotype, biotype, and β -lactamase production of respiratory isolates of *Haemophilus influenzae*. Am J Clin Pathol. 1984 Jan;81(1):85–88. <https://doi.org/10.1093/ajcp/81.1.85>
- Butler DE, Myers AL. Changing epidemiology of *Haemophilus influenzae* in children. Infect Dis Clin North Am. 2018 Mar;32(1):119–128. <https://doi.org/10.1016/j.idc.2017.10.005>
- Cherkaoui A, Diene SM, Emonet S, Renzi G, Francois P, Schrenzel J. Ampicillin-resistant *Haemophilus influenzae* isolates in Geneva: serotype, antimicrobial susceptibility, and β -lactam resistance mechanisms. Eur J Clin Microbiol Infect Dis. 2015 Oct;34(10):1937–1945. <https://doi.org/10.1007/s10096-015-2435-5>
- CLSI. Performance standards for antimicrobial susceptibility testing. 21st ed. CLSI supplement M100-S21. Wayne (USA): Clinical and Laboratory Standards Institute; 2021.
- Collins JK, Kelly MT. Comparison of Phadebact coagglutination, Bactogen latex agglutination, and counterimmunoelectrophoresis for detection of *Haemophilus influenzae* type b antigens in cerebrospinal fluid. J Clin Microbiol. 1983 Jun;17(6):1005–1008. <https://doi.org/10.1128/jcm.17.6.1005-1008.198>
- Crandall H, Christiansen JE, Varghese A, Russon AK, Korgenski K, Dickey M, Killpack J, Knackstedt E, Daly J, Ampofo K, et al. 1522. Clinical and molecular epidemiology of invasive *Haemophilus influenzae* serotype a infections in Utah children. Open Forum Infect Dis. 2019 Oct;6(Supplement_2):S554. <https://doi.org/10.1093/ofid/ofz360.1386>
- Dong Q, Shi W, Cheng X, Chen C, Meng Q, Yao K, Qian S. Wide-spread of non-typeable *Haemophilus influenzae* with high genetic diversity after two decades use of Hib vaccine in China. J Clin Lab Anal. 2020 Apr;34(4):e23145. <https://doi.org/10.1002/jcla.23145>
- Falla TJ, Crook DW, Brophy LN, Maskell D, Kroll JS, Moxon ER. PCR for capsular typing of *Haemophilus influenzae*. J Clin Microbiol. 1994 Oct;32(10):2382–2386. <https://doi.org/10.1128/jcm.32.10.2382-2386.1994>
- Fally M, Israelsen S, Anhøj J, Benfield T, Tarp B, Kolte L, Ravn P. The increasing importance of *Haemophilus influenzae* in community-acquired pneumonia: results from a Danish cohort study. Infect Dis. 2021 Feb;53(2):122–130. <https://doi.org/10.1080/23744235.2020.1846776>
- Fu P, He L, Wang C, Yu H, Xu H, Jing C, Deng J, Zhao R, Hua C, Chen Yu, et al. [Antimicrobial resistance profile of clinical isolates in pediatric hospitals in China: report from the ISPED Surveillance Pro-

- gram in 2019] (in Chinese). *Chin J Evidence-Based Pediatr*. 2021 Feb; 16(1):43–50. <https://doi.org/10.3969/j.issn.1673-5501.2021.01.002>
- Fu P, Xu H, Jing C, Deng J, Wang H, Hua C, Chen Y, Chen X, Zhang T, Zhang H, et al. Bacterial epidemiology and antimicrobial resistance profiles in children reported by the ISPED program in China, 2016 to 2020. *Microbiol Spectr*. 2021 Dec;9(3):e0028321. <https://doi.org/10.1128/Spectrum.00283-21>
- Fuji N, Pichichero M, Kaur R. *Haemophilus influenzae* prevalence, proportion of capsulated strains and antibiotic susceptibility during colonization and acute otitis media in children, 2019–2020. *Pediatr Infect Dis J*. 2021 Sep;40(9):792–796. <https://doi.org/10.1097/INF.0000000000003171>
- Heinz E. The return of Pfeiffer's bacillus: rising incidence of ampicillin resistance in *Haemophilus influenzae*. *Microb Genom*. 2018 Sep;4(9):e000214. <https://doi.org/10.1099/mgen.0.000214>
- Heliodoro CIM, Bettencourt CR, Bajanca-Lavado MP; Portuguese Group for the Study of *Haemophilus influenzae* invasive infection. Molecular epidemiology of invasive *Haemophilus influenzae* disease in Portugal: an update of the post-vaccine period, 2011–2018. *Eur J Clin Microbiol Infect Dis*. 2020 Aug;39(8):1471–1480. <https://doi.org/10.1007/s10096-020-03865-0>
- Kilian M, Sørensen I, Frederiksen W. Biochemical characteristics of 130 recent isolates from *Haemophilus influenzae* meningitis. *J Clin Microbiol*. 1979 Mar;9(3):409–412. <https://doi.org/10.1128/jcm.9.3.409-412.1979>
- Kilian M. *Haemophilus*. In: Lennette EH, Balows A, Hausler WJ, Shadomy HJ, editors. *Manual of clinical microbiology*, 4th ed. Washington (USA): American Society for Microbiology; 1985. p. 387–393.
- Kim IS, Ki CS, Kim S, Oh WS, Peck KR, Song JH, Lee K, Lee NY. Diversity of ampicillin resistance genes and antimicrobial susceptibility patterns in *Haemophilus influenzae* strains isolated in Korea. *Antimicrob Agents Chemother*. 2007 Feb;51(2):453–460. <https://doi.org/10.1128/AAC.00960-06>
- Langereis JD, de Jonge MI. Unraveling *Haemophilus influenzae* virulence mechanisms enable discovery of new targets for antimicrobials and vaccines. *Curr Opin Infect Dis*. 2020 Jun;33(3):231–237. <https://doi.org/10.1097/QCO.0000000000000645>
- Li XX, Xiao SZ, Gu FF, He WP, Ni YX, Han LZ. Molecular epidemiology and antimicrobial resistance of *Haemophilus influenzae* in adult patients in Shanghai, China. *Front Public Health*. 2020 Mar;8:95. <https://doi.org/10.3389/fpubh.2020.00095>
- Nørskov-Lauritsen N, Pedersen N, Lam JUH, Nielsen HL, Kobel CM, Hansen DS; ODiD Consortium. *Haemophilus influenzae* one day in Denmark: prevalence, circulating clones, and dismal resistance to aminopenicillins. *Eur J Clin Microbiol Infect Dis*. 2021 Oct;40(10):2077–2085. <https://doi.org/10.1007/s10096-021-04247-w>
- Schotte L, Wautier M, Martiny D, Piérard D, Depypere M. Detection of beta-lactamase-negative ampicillin resistance in *Haemophilus influenzae* in Belgium. *Diagn Microbiol Infect Dis*. 2019 Mar; 93(3):243–249. <https://doi.org/10.1016/j.diagmicrobio.2018.10.009>
- 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 Jun;22(4):247–252. <https://doi.org/10.1089/mdr.2015.0189>
- Ubukata K, Morozumi M, Sakuma M, Adachi Y, Mokuno E, Tajima T, Iwata S; AOM Surveillance Study Group. Genetic characteristics and antibiotic resistance of *Haemophilus influenzae* isolates from pediatric patients with acute otitis media after introduction of 13-valent pneumococcal conjugate vaccine in Japan. *J Infect Chemother*. 2019 Sep;25(9):720–726. <https://doi.org/10.1016/j.jiac.2019.03.019>
- Vallejo JG, McNeil JC, Hultén KG, Sommer LM, Dunn JJ, Kaplan SL. Invasive *Haemophilus influenzae* disease at Texas Children's Hospital, 2011 to 2018. *Pediatr Infect Dis J*. 2019 Sep;38(9): 900–905. <https://doi.org/10.1097/INF.0000000000002383>
- Wen S, Feng D, Chen D, Yang L, Xu Z. Molecular epidemiology and evolution of *Haemophilus influenzae*. *Infect Genet Evol*. 2020 Jun; 80:104205. <https://doi.org/10.1016/j.meegid.2020.104205>
- Yamada S, Seyama S, Wajima T, Yuzawa Y, Saito M, Tanaka E, Noguchi N. β -Lactamase-non-producing ampicillin-resistant *Haemophilus influenzae* is acquiring multidrug resistance. *J Infect Public Health*. 2020 Apr;13(4):497–501. <https://doi.org/10.1016/j.jiph.2019.11.003>
- Zhou Y, Wang Y, Cheng J, Zhao X, Liang Y, Wu J. Molecular epidemiology and antimicrobial resistance of *Haemophilus influenzae* in Guiyang, Guizhou, China. *Front Public Health*. 2022 Dec 1;10:947051. <https://doi.org/10.3389/fpubh.2022.947051>