Infection and Drug Resistance

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ORIGINAL RESEARCH

High Prevalence of Group III-Like Mutations Among BLPACR and First Report of *Haemophilus influenzae* ST95 Isolated from Blood in China

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Purpose: We aimed to evaluate antibiotic resistance and molecular epidemiological characteristics of non-invasive *Haemophilus influenzae* (*H. influenzae*) from pneumonia patients and analyze the whole genome of one invasive *H. influenzae* isolated from blood in pediatric patients.

Methods: Antibiotic susceptibility was tested using the turbidimetric method. β-lactamase-producing and serotyping genes were evaluated via multiplex polymerase chain reaction (PCR), and *ftsI* was amplified using high-fidelity PCR. Lastly, whole genome sequencing (WGS) was conducted using Illumina HiSeq and PacBio sequencing technology.

Results: We observed that the ampicillin (AMP) and amoxicillin/clavulanate (AMC) resistance rates of non-invasive *H. influenzae* were as high as 99.06% (after adjustment) and 49.53%, respectively. The β-lactamase gene of 106 AMP-resistant strains was bla_{TEM-1}. Group III-like mutation accounted for 71.15% of β-lactamase-positive, AMC-resistant (BLPACR) strain mutants. The novel Asn-526→His mutation was present in one β-lactamase-negative AMP-susceptible (BLNAS) strain. Non-invasive *H. influenzae* strains all belonged to non-typeable *H. influenzae* (NTHi). In contrast, the invasive *H. influenzae* 108 isolated from blood in China belonged to *H. influenzae* type b (Hib). It belonged to sequence typing ST95 and exhibited sensitivity to all 11 antibiotics. Three prophages were identified, and the *cap*b loci of the *H. influenzae* strain 108 revealed regions I–III exist in duplicate; however, complete deletion of IS1016 was only present in one of the copies.

Conclusion: Non-invasive *H. influenzae* NTHi with β-lactamase-positive was highly prevalent. Notably, group III-like mutations had increased prevalence among BLPACR strains. *H. influenzae* belonging to Hib and ST95 was first reported to cause sepsis in China.

Keywords: group III-like mutations, BLPACR, prevalence, invasive *Haemophilus influenzae*, genome

Introduction

H. influenzae, a gram-negative *bacillus* that commonly colonizes the nasopharynx of humans, is an opportunistic pathogen that can cause upper respiratory tract infection, pneumonia, asthma, other respiratory diseases, and severe systemic infections such as purulent meningitis and sepsis.[1,2] Invasive *H. influenzae* disease is defined as a *H. influenzae* infection isolated from sterile sites such as blood, pleural fluid, peritoneal fluid, cerebrospinal fluid etc. Children under five years of age, especially those under one year of age, are more likely to develop invasive *H. influenzae* disease. It can also occur in individuals without any underlying diseases. It is more often severe, with a high mortality rate or various sequelae left in survivors.[2,3] Non-invasive *H. influenzae* disease has a better prognosis; however, the disease burden remains high owing to its high occurrence in children.

Received: 4 December 2022
Accepted: 14 February 2023
Published: 17 February 2023

H. influenzae is divided into two types: encapsulated type and non-encapsulated type. There are six serotypes: a, b, c, d, e, and f, often abbreviated as Hia, Hib, Hic, Hid, Hie, and Hif, respectively. NTHi is non-encapsulated and does not agglutinate with either type of serum. In a few cases, the encapsulated strains ceased producing capsules to adapt to the environment. Therefore, simple serotyping will be misjudged as NTHi, and capsular gene testing is required. In recent years, Hib infections have become rare with the widespread introduction of the Hib vaccine. Previous population-based studies in Hefei and Nanning, China, found that the incidence of Hib meningitis was 10.66 and 0.98 cases/100,000 children <5 years of age in the pre- and post-vaccine eras, respectively.[4,5] However, the proportion of Hia and Hif in the infected population in some regions has increased,[3,6] and NTHi has become the most common pathogen of *H. influenzae* disease globally.[7] The gene sequence IS1016 has been associated with an increased capacity to cause severe infections. It is usually present in encapsulated strains but is sometimes harbored by NTHi.[8] In nasopharyngeal colonization, NTHi can easily exchange large DNA fragments between strains. This exchange is done to obtain new virulence factors to enhance its ability to resist human neutrophils and complement-mediated bactericidal effects.[9,10] Consequently, NTHi is already the most common cause of invasive *H. influenzae* disease in children under one year of age.[11]

The preferred drugs for children infected with *H. influenzae* include oral ampicillin (AMP), amoxicillin/clavulanate (AMC), and an intravenous infusion of cefotaxime (CTX) and ceftriaxone. With the widespread use of antibiotics, the increasing trend of the *H. influenzae* resistance rate to various antibacterial drugs has received extensive clinical attention. There are two main mechanisms of β-lactam antibiotic resistance in *H. influenzae*. One mechanism is the inactivation of AMP by hydrolysis of β-lactamase (TEM-1 or ROB-1 type encoded by $bla_{TEM\text{-}1}$ or $bla_{ROB\text{-}1}$ gene, respectively). *H. influenzae* with β-lactamase-positive AMP resistance is called β-lactamase-positive, AMP-resistant (BLPAR). The other mechanism is that an amino acid site mutation of the penicillin-binding protein (PBP3) encoded by *ftsI* causes changes in the spatial conformation, which reduces its affinity for AMP. *H. influenzae* with *ftsI* mutation for AMP resistance without producing β-lactamase is called β-lactamase negative, AMP resistant (BLNAR).[12] AMC-resistant strain with β-lactamase-positive is called β-lactamase-producing AMC-resistant (BLPACR), often occurring in *ftsI* mutations.[13]

A recent multicenter study in China showed that the resistance rates of *H. influenzae* to AMP was increasing year by year, and there were statistically significant differences in the resistance rates of all antibiotics in different regions except chloramphenicol.[14] Since the introduction of the Hib vaccine in Fujian, China, in 2003, there has been no research on antibiotic resistance and molecular epidemiological characteristics of *H. influenzae*. Therefore, in this study, we investigated the antibiotic resistance and molecular characterization of *H. influenzae* isolated from pediatric children in Quanzhou, China, to provide a basis for the prevention and treatment of *H. influenzae* disease.

Materials and Methods

Strains

A total of 108 *H. influenzae* cases were collected from hospitalized children in Quanzhou Women's and Children's Hospital from July 2021 to August 2022. The mean age of the children was 1.56±0.16 years. There were 77 boys and 31 girls, and the sex ratio was 2.48. The non-invasive *H. influenzae* inclusion criteria were <10 squamous epithelium and >25 white blood cells (WBC) in each low-power field of view to avoid the collection of colonized *H. influenzae*. Gram-negative *bacilli* were seen in the WBC. In addition, there were evident clinical features of *H. influenzae* community-acquired pneumonia. Invasive *H. influenzae* inclusion criteria was that *H. influenzae* was cultured from double blood culture bottles. Repeated isolates from the same infection sites of the same patients were excluded. Among the 108 *H. influenzae* strains, 106 (98.15%) non-invasive strains were isolated from sputum, one (0.92%) non-invasive strain from bronchoalveolar lavage fluid, and one (0.92%) named invasive *H. influenzae* 108 from the blood of an unvaccinated pediatric patient. This pediatric patient was clinically characterized by fever, headache, and vomiting. During the first three days of hospitalization, ceftazidime (CAZ) was used for anti-infection. According to 1.151×10^9/L WBC in cerebrospinal fluid, positive culture for *H. influenzae* and the 135.12 mg/L value of C-reactive protein, vancomycin + meropenem were given for the next 3 days. After 6 days of treatment, the symptoms improved significantly and he was discharged from the hospital. *H. influenzae* strains were stored at −80°C in BHI-sheep blood-20% glycerol storage

solution. This study complied with the Declaration of Helsinki, and ethical permission for this study was approved by the Quanzhou Women's and Children's Hospital ethics committee (2021 Ethical Review No. 16). Guardians provided written informed consent on behalf of the minors. *H. influenzae* strains were identified by VITEK 2 bioMérieux bacterial identification instrument. *H. influenzae* ATCC 49247 was the quality control strain.

Antibiotic Susceptibility Test

Antibiotic susceptibility testing was performed using the turbidimetric method (similar to microdilution) of the ATB HAEMO CLSI (12) strip from bioMérieux. Antibiotics that were evaluated include AMP, AMC, Cefoxitin (CEP), Cefaclor (CEC), CAZ, Cefotaxime (CTX), Ofloxacin (OFX), Rifampicin (Rif), Chloramphenicol (CHL), Tetracycline (TCY), and Cotrimoxazole (SXT). First, *H. influenzae* colonies were cultured for 24 hours, and the bacterial suspension was adjusted to 0.5 McF concentration (0.85% NaCl). Subsequently, 50 µL of bacterial suspension was transferred to ATB S medium. After mixing, 135 µL of suspension was added to each well of the ATB HAEMO CLSI (12) reagent strip. The strip was then placed in a CO_2 incubator for 20 h. Finally, the results were read automatically using an ATB instrument. The results were interpreted based on the breakpoints recommended by the CLSI M100-S23 2013 committee for *H. influenzae*. The quality control strain used was *H. influenzae* ATCC 49247.

Detection of β-Lactamase-Producing Genes and Serotype Capsular Genes

β-lactamase-producing genes and serotype capsular genes were assessed by the Multiplex PCR method. Bacterial DNA was extracted according to the instructions of the TIANamp Bacteria DNA Kit. The PCR amplification primer sequences and product lengths of β-lactamase-producing genes (bla_{TEM-1} and bla_{ROB-1}) and serotype capsular genes (a–f) are listed in Table S1. Multiplex PCR reagents were purchased from Vazyme. The total reaction volume of 50 µL consisted of 7 µL RNase-free ddH_2O, 25 µL 2× Multiplex buffer, 5 µL 10× primer mix (Premix all amplification primers so that the concentration of each primer is 1 µM), 2 µL template DNA, 1 µL Multiplex DNA polymerase, and 10 µL 5× Multiplex GC Enhancer. The thermal cycle was programmed for 5 min at 95°C for pre-denaturation, followed by 35 cycles of 30s at 95°C for denaturation, 90s at 60°C for annealing, and 90s at 72°C for extension; a final extension was conducted for 10 min at 72°C. The PCR products were observed using agarose gel electrophoresis.

Amplification and Sequencing of *fstI* Gene

The *ftsI* gene was amplified by high-fidelity PCR. The PCR amplification primer sequences and product lengths of *ftsI* are listed in Table S1. High-fidelity PCR was purchased from Vazyme. The total reaction volume of 50 µL consisted of 20 µL ddH_2O, 25 µL 2× Phanta Max Master Mix, 2 µL primer F, 2 µL primer R, and 1 µL template DNA. The thermal cycle was programmed for 3 min at 95°C for pre-denaturation, followed by 32 cycles of 15s at 95°C for denaturation, 15s at 58°C for annealing, 60s at 72°C for extension; a final extension was conducted for 5 min at 72°C. The PCR products were observed using agarose gel electrophoresis.

Subsequently, the PCR products were sent to Fuzhou Shangya Biological Co., Ltd. for Sanger sequencing. Amino acids 330–530 of PBP3, including the highly conserved motifs Ser-Thr-Val-Lys (STVK), Lys-Thr-Gly (KTG), and Ser-Ser-Asn (SSN), were compared with the corresponding sequence of *H. influenzae* Rd KW20. The PBP3 mutation patterns were determined according to the rules reported in the literature:[15] Group I was defined as Arg-517→His-517. Group II as Asn-526→Lys-526). Group II was further divided into four subgroups. IIa: only Asn-526→Lys-526; IIb: Asn-526→Lys-526 and Ala-502→Val-502; group IIc: Asn-526→Lys-526 and Ala-502→Thr-502; group IId: Asn-526→Lys-526 and Ile-449→Val. Group III was defined as Met-377→Ile, Ser-385→Thr, Leu-389→Phe, and Asn-526→Lys). Group III-like was defined as Met-377→Ile, Ser-385→Thr, and/or Leu-389→Phe in the SSN motif, Arg-517→His and Thr-532→Ser in the KTG motif, Asp-350→Asn in the STVK motif; and finally, Ser-357→Asn.

Whole Genome Sequencing (WGS)

The *H. influenzae* 108 isolated from blood was sent to Majorbio Bio-Pharm Technology Co., Ltd. for WGS. In this study, whole genome de novo sequencing was performed using the second-generation Illumina HiSeq and third-generation PacBio sequencing methods. No less than 100× Illumina sequencing data of the genome and 100× PacBio sequencing

data were provided to ensure a complete and accurate assembly while avoiding the loss of small plasmid (<15 kb) information. Further, this information ensured that the complete genome contained the plasmid. The genome coverage of *H. influenzae* 108 strain was 100% for this sequencing. The Hicap tool (https://github.com/scwatts/hicap), online bioinformatic tools (http://www.genomicepidemiology.org/), the comprehensive antibiotic resistance database (https://card.mcmaster.ca/), and Abricate v.0.8 (https://github.com/tseemann/abricate) with the VFDB database (http://www.mgc.ac.cn/Vfs/) were used to analyze the serotypes, sequence types, resistomes, and virulomes. A >80% for gene identity was considered as the threshold for predicting virulence genes. In addition, PHASTER (https://phaster.ca/) was used to predict the prophage regions. A CLC sequence viewer was used to investigate single nucleotide polymorphisms (SNPs) of the cap loci, the complete deletion of the putative virulence-enhancing IS1016, and the partial deletion of *bexA*. 16S rRNA was utilized for performing phylogenetic analyses using megax software. The evolutionary relationship was inferred using maximum likelihood method.

Nucleotide Sequence

The complete sequences of *H. influenzae* 108 was deposited in GenBank under the accession number: PRJNA900629.

Results

Analysis of Drug Resistance in *H. influenzae*

We observed that the AMP resistance rate of non-invasive *H. influenzae* was as high as 91.59%, followed by the SXT resistance rate of 71.96%, CEC of 60.74%, CAZ of 56.07%, AMC of 49.53%, and CEP of 46.73%. CTX, OFX, Rif, and CHL were not resistant, as presented in Table 1. Only 2 non-invasive *H. influenzae* strains (1.87%) showed multidrug-resistance (MDR). Their MDR pattern was resistance to β-lactam antibiotics (AMP, AMC, CEP, CEC, CAZ), TCY and SXT. One BLNAS strain and the invasive *H. influenzae* 108 was sensitive to all 11 antibiotics.

Analysis of β-Lactamase-Producing Genes in 107 Non-Invasive *H. influenzae* Strains

The β-lactamase gene of 98 AMP-resistant strains were all bla_{TEM-1} genes but not bla_{ROB-1}. BLNAR strains were not detected. The instructions of the *H. influenzae* drug susceptibility test kit recommend verifying the AMP-susceptible strains to confirm the absence of β-lactamase. Therefore, the β-lactamase-producing gene of AMP-sensitive strains was detected. Notably, the bla_{TEM-1} gene was detected in eight of the nine isolates. Only one strain was confirmed as β-lactamase-producing gene negative. Finally, the AMP resistance rate was corrected to 99.06%.

Table 1 Antibiotic Resistance of 107 Non-Invasive *H. influenzae* Strains

Antibiotics	Number of Resistant Bacteria	Resistance Rate (%)	Intermediary Rate (%)	Sensitivity Rate (%)
AMP	98	91.59	0	8.41
AMC	53	49.53	0	50.47
CEP	50	46.73	0	53.27
CEC	65	60.74	3.75	35.51
CAZ	60	56.07	1.87	42.06
CTX	0	0	0	100
OFX	0	0	0.93	99.07
Rif	0	0	0	100
CHL	0	0	0	100
TCY	2	1.87	0	98.13
SXT	77	71.96	0.94	27.10

Mutation Analysis of *ftsI* in 53 BLPACR Strains and One BLNAS Strain, and Serotype Analysis of 107 Non-Invasive *H. influenzae* Strains

We identified eight mutational patterns from 52 BLPACR strains; however, one BLPACR strain expressed no mutations (Table 2). Thirty-seven strains were group III-like, accounting for 71.15% of BLPACR strain mutants. Eleven strains belonged to group III, of which four were combined with group IIb or group IIc. Two strains belonged to group IIc. The untyped patterns of the two BLPACR strains were Asp-350→Asn, Ser-357→Asn, Met-377→Ile, and Ser-385→Thr. In one BLNAS strain, the mutation pattern was Asp-350→Asn, Ser-385→Thr, Leu-389→Phe, and Asn-526→His.

All 107 non-invasive *H. influenzae* strains belonged to NTHi because the six serotype genes were negative.

Whole Genome Analysis of Invasive *H. influenzae* 108

WGS of invasive *H. influenzae* 108 revealed the genome size to be 1,903,516 bp, comprising 1798 protein-coding sequences, 20 rRNAs, and 57 tRNAs, with a GC content of 38.1%. No plasmids were present in the strains. Three prophages were identified: one intact, two questionable (Figure 1). The prophages 1 (questionable), located at 478,683–513,213, is 34.5 Kb in length and contains 54 proteins. The prophages 2 (intact), located at 1,089,401–1,145,733, is 56.3 Kb in full length and contains 61 proteins. The prophages 3 (questionable), located at 1,303,040–1,319,103, is 16 Kb in full length and contains 22 proteins. No exogenous antimicrobial resistance or putative virulence genes were present in the prophage regions.

The invasive *H. influenzae* 108 were discovered to belong to Hib, and its sequence type belonged to ST95. β-lactamase-producing genes and *ftsI* mutations were not detected. However, the *LpsA* gene for intrinsic peptide antibiotic resistance and the EF-Tu R234F amino acid mutation, which mediates felfamycin resistance, were present. According to the analysis of 16S rRNA phylogenetic tree, *H. influenzae* 108 was the closest to *H. influenzae* NCTC11394 and *H. influenzae* M3918 (Figure 2).

Comparative analysis of the *cap*b loci of the *H. influenzae* 108 and the *H. influenzae* 146 references (GenBank accession number: PQJI00000000.1) revealed regions I, II, and III exist in duplicates. However, IS1016 complete deletion rather than partial deletion was identified upstream of the region I in only one of the duplicate copies (Figure 3). Compared with the multiple mutations in the SNPs analysis of the *cap*b locus from reference *H. influenzae* 146, no mutations were identified in the *H. influenzae* 108.

There are 79 predicted related virulence genes, as displayed in Table S2. These virulence genes can be divided into four categories: capsule gene (20 genes), lipopolysaccharide (LPS) biosynthesis-related genes (45 genes), nonspecific virulence factors (6 genes), defensive virulence factors (3 genes), and offensive virulence factors (5 genes).

Table 2 Different Amino Acid Substitution Patterns Identified in Part of PBP3 from 53 BLPACR Strains and One BLNAS Strain

BLPACR	BLNAS	Amino Acid Substitution Forms											
No	No	Asp-350	Ser-357	Met-377	Ser-385	Leu-389	Ile-449	Gly-409	Ala-502	Arg-517	Asn-526	Thr-532	group
23		Asn	Asn	Ile	Thr	Phe				His		Ser	III like
14		Asn	Asn	Ile	Thr					His		Ser	III like
2		Asn	Asn	Ile	Thr	Phe			Val		Lys		IIb+III
2		Asn	Asn		Thr			Glu	Thr		Lys		IIc
2		Asn	Asn	Ile	Thr	Phe			Thr		Lys		IIc+III
5		Asn	Asn	Ile	Thr	Phe					Lys		III
2		Asn	Asn	Ile	Thr	Phe		Glu			Lys		III
2		Asn	Asn	Ile	Thr								Untyped
	1	Asn			Thr	Phe					His		Untyped
1													–

Note: –, No mutation found.

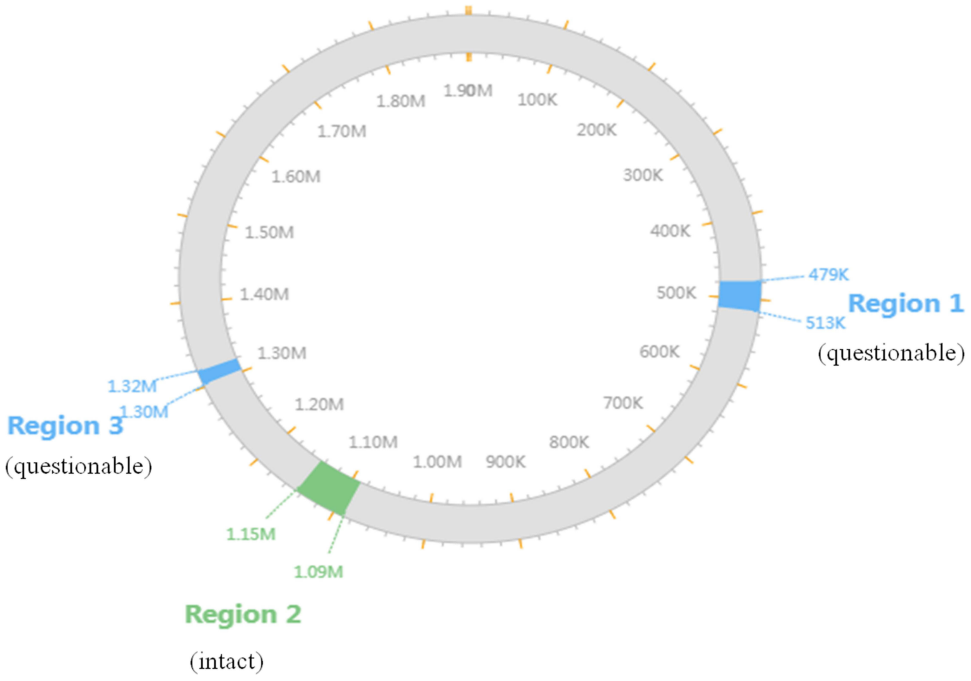

Figure 1 The locations of the prophages on the chromosome of the strain Regions 1 to 3 represent prophages 1 to 3; Blue, red and green represent questionable, incomplete, and intact, respectively.

Discussion

H. influenzae, a common pathogen of community-acquired pneumonia, was majority isolated from the sputum of the lower respiratory tract. In this study, we observed a 99.06% AMP resistance rate, which was higher than the 61.4% reported by a Chinese multicenter study in 2016,[16] and the 13% in Paraguay[17] but similar to the 91.1% reported by another Chinese study in 2022.[18] This increasing trend may be attributed to the 99.06% detection rate of the β-lactamase gene in this study. Except for AMC, the resistance rates of CEC, CTX, OFX, Rif, TCY, and SXT were consistent with those reported in another Chinese study in 2022.[18] Compared with other reports of CHL low resistance,[18] no CHL resistance was observed in this study. A multi-center study in China showed that the major multidrug resistance pattern was resistant to β-lactams, macrolides, and sulfonamides,[14] which was inconsistent with the MDR pattern in this study. The MDR detection rate in this study was much lower than 12.8% reported in China.[19] MDR may exhibit a unique resistance pattern in a low prevalence state at Quanzhou.

Given that CHL, OFX, and TCY are contraindicated in clinical use for pediatric patients, CTX and Rif, with a 100% sensitivity rate, can be the first choice for empirical medication. The high AMC resistance rate of 49.53% observed in this study was associated with the reduced drug sensitivity caused by the high *PBP3* mutation rate. Therefore, it is not recommended that AMC be the drug of choice for treatment.

Chromosome-mediated overproduction of dihydrofolate reductase is the primary resistance mechanism of SXT. SXT resistance rate is also high, close to 72%; therefore, it is not recommended for empirical treatment.

The β-lactamase genes identified in this study were all bla_{TEM} genes, consistent with a previous Chinese study.[20] Furthermore, eight strains carrying β-lactamase-producing $bla_{TEM\text{-}1}$ genes were detected from nine AMP-sensitive strains to clarify the detection efficiency of the ATB HAEMO CLSI (12) strip for AMP sensitivity. The missed detection rate was 8/107 (7.48%), which exhibits effective false negative prevention.

The primary resistance-conferring mutations are in the transpeptidase domain of PBP3. These mutations decrease the binding of β-lactam antibiotics to PBP3 under the selective pressures of different β-lactam antibiotics.[21,22] BLNAR strains had reduced sensitivity to AMC and its combinations with beta-lactamase inhibitors and second-generation cephalosporins.[23] However, no BLNAR strains were identified in this study. The detection rate of BLPACR strains was 49.53%, which was higher than the 15.4% reported in another Chinese study,[24] 23% in a British Columbian study,[25]

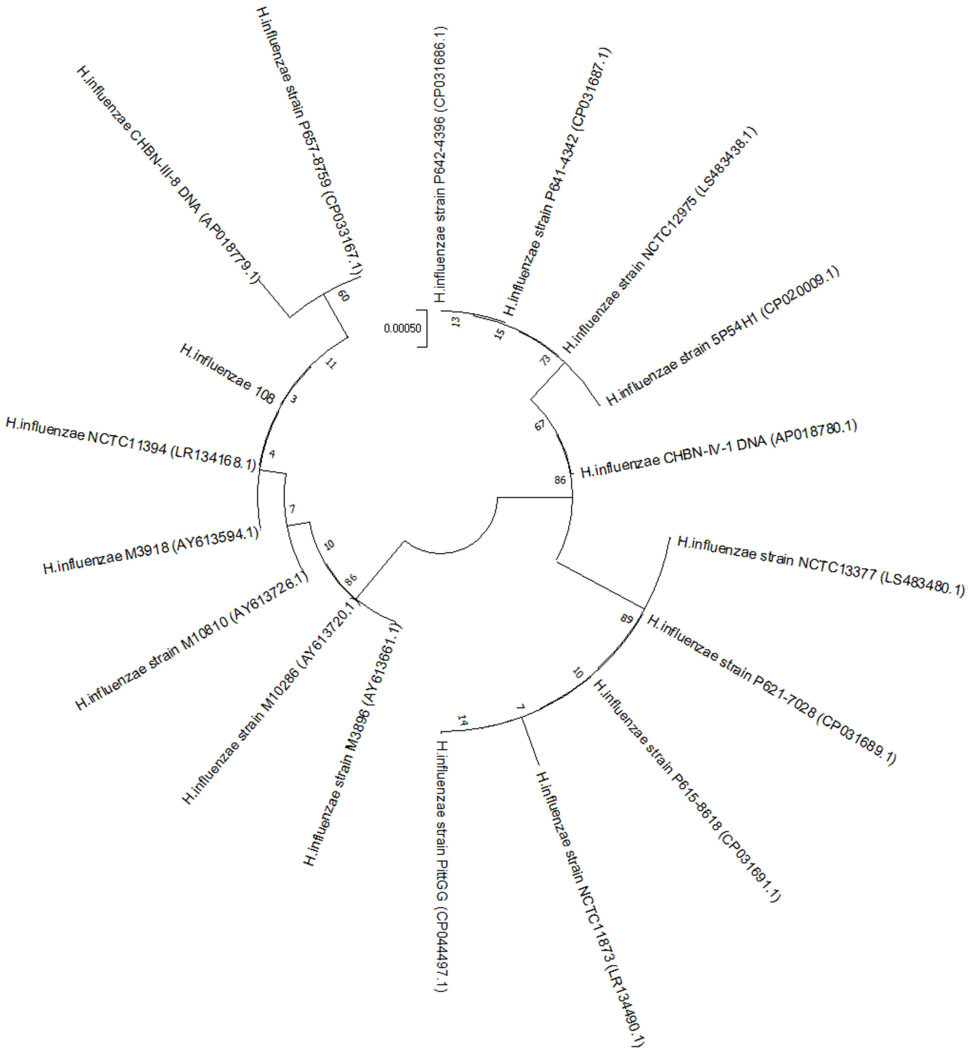

Figure 2 Phylogenetic analysis between the *H. infuenzae* strains The 17 *H. infuenzae* genomes available on the NCBI database and *H. influenzae* strain 108 isolated in this study.

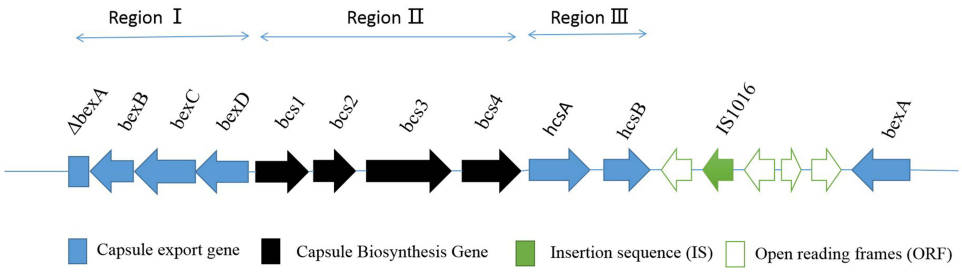

Figure 3 Cap locus structure identified in *H. influenzae* strain 108 Arrows indicate the direction of transcription and translation, and squares indicate the deletion of a part of the gene.

and 32.1% in a Korean study.[26] The MICs of CTX for group III-like strains were at least 10 times higher than fully susceptible isolates.[22] Moreover, group III and III-like *H. influenzae* strains were more resistant to β-lactam antibiotics than group I and II strains.[26,27] BLPACR strains were the main group III and III-like strains in this study, which is consistent with previous literature.[26,28] However, group III-like BLPACR strains exhibited a high prevalence. One BLPACR strain was observed to have no variation in the amino acid sequence 350–532 of PBP3. We speculated that

this strain might carry resistance mutations in other regions of PBP3 or other β-lactam resistance genes. Asn-526→His in the KTG motif, with Asp-350→Asn, Ser-385→Thr, and Leu-389→Phe were first reported in one BLNAS strain, which may slightly reduce or not affect the MIC values of β-lactam antibiotics.

With the introduction of the *H. influenzae* vaccine, the serotypes of strains causing respiratory diseases are mainly NTHi.[28] In this study, we observed that all non-invasive strains were NTHi, consistent with the strain isolated from the lower respiratory tract previously reported in China.[29] However, BLPACR NTHi strains are highly prevalent in Korea.[26] We also observed that BLPACR NTHi strains were popular in China, thereby drawing attention to the increasing β-lactam resistance.

Compared with the reference genome of *H. influenzae* 146 belonging to Hib, this *H. influenzae* 108 strain's genome is relatively large but does not carry a plasmid containing the $bla_{TEM\text{-}1B}$ gene and has no mutations in *ftsI*, which may explain the lack of β-lactam antibiotic resistance. Diversification due to genome rearrangement has been reported to contribute to the global spread of *H. influenzae*.[30] Compared with the strains reported to carry four complete prophages and one incomplete prophage,[31] the strains in this study carried fewer prophages, resulting in less phenotype diversity. In the pre-vaccination era, *H. influenzae* ST95, which belonged to Hib and was transferred by healthy Japanese children, exhibited resistance to AMP and TCY.[32] In the post-Hib vaccine era, some invasive *H. influenzae* Hib and ST-95 isolated from Canada exhibited no resistance, whereas some demonstrated resistance to AMP owing to β-lactamase production.[33] *H. influenzae* Hib and ST-95 isolated from children with bacterial meningitis are typical for strains circulating in Russia.[34] *H. influenzae* Hib and ST-95 have been isolated from the respiratory tract in China.[35] In this study, we identified *H. influenzae* Hib and ST-95 isolated from Chinese children's blood for the first time, which demonstrated no resistance to eleven antibiotics. *H. influenzae* NCTC11394 and *H. influenzae* M3918 isolated from USA also belonged to Hib, which is the same as *H. influenzae* 108 serotype. Although *H. influenzae* NCTC11394 belonged to ST6 through MLST analysis by us, the scores of 6 of the 7 housekeeping genes of its ST were consistent with those of *H. influenzae* 108 and *H. influenzae* 108 belonged to ST-6 complex. This further indicates that the two strains are related in evolution.

The capsular polysaccharide of *H. influenzae* Hib is a major virulence factor and a crucial antigen for Hib vaccination.[36] The *cap*b locus, which consists of three functionally distinct regions (I to III), is responsible for generating the type b capsule.[37] Multiple copies of the *cap*b locus exhibit strong virulence and can result in increased polysaccharide production. Another large category of virus-related genes are those involved in LPS synthesis.[38] The LPS of *H. influenzae* may play an important role in the pathogenesis of meningitis by destroying the blood-brain barrier[39] and septicemia.[40] The strains in this study also comprised invasiveness LPS biosynthesis genes, *lic1* and *lic2*, consistent with Cardosoa et al, suggesting that these genes exist in invasive strains.[37] We also observed that *H. influenzae* Hib and ST-95 caused severe septicemia in unvaccinated children, highlighting the indispensable and crucial role of vaccines.

Conclusions

In this study, AMP and AMC were not suitable for empiric medication due to high drug resistance rate. MDR was in a very low prevalence state at this region. BLPACR NTHi strains dominated by group III-like showed high prevalence. *H. influenzae* Hib and ST-95 was isolated from blood for the first time in China. In addition, genomic surveillance study conducted to monitor changes in the molecular epidemiology of invasive *H. influenzae* infection was important.

Acknowledgments

We expressed our appreciation to the patient and her parents in this study.

Funding

This research was funded by Quanzhou Municipal Science and Technology Plan Project (Grant No: 2021N089S).

Disclosure

The authors declare that they have no competing interests.

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