



Characteristics and Antibiotic Resistance of *Haemophilus influenzae* in Children with Lower Respiratory Tract Infection in Chengdu, China

Qin Wang ^{1,*}, Cheng-Gui Liu¹, Jian Xu¹, Qin Zhang¹, Chong-Hui Zhao¹ and Pei-Pei Song¹

¹Department of Clinical Laboratory, Chengdu Women's and Children's Central Hospital, School of Medicine, University of Electronic Science and Technology of China, Chengdu 611731, China

*Corresponding author: Department of Clinical Laboratory, Chengdu Women's and Children's Central Hospital, School of Medicine, University of Electronic Science and Technology of China, Chengdu 611731, China. Email: cqwq1986925@sina.com

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Abstract

Background: *Haemophilus influenzae* is an opportunistic pathogen of the human respiratory tract. *Haemophilus influenzae* can cause not only respiratory tract infection in children but also otitis media, epiglottitis and sinusitis. With the widespread use of antibiotics, the positive rate of β -lactamase in *H. influenzae* is increasing, and the rate of antimicrobial resistance is also increasing, which increases the difficulty of clinical treatment.

Objectives: To study the infection characteristics of patients and the antibiotic resistance of *H. influenzae* in lower respiratory tract samples of children in Chengdu, so as to provide a reference for its clinical diagnosis and the rational use of antibiotics.

Methods: Sputum samples of 15891 children aged 0-14 years with lower respiratory tract infection were collected. *Haemophilus influenzae* was cultured and identified, its drug susceptibility tested, and the results determined according to the guidelines of CLSI 2020.

Results: A total of 15891 clinical isolate strains in sputum were detected for drug sensitivity from December 2018 to January 2020, of which 5488 were *H. influenzae*, accounting for 34.54% (5488/15891). The sex of children infected with *H. influenzae* was not skewed ($P > 0.05$). The detection rate of *H. influenzae* was the highest in children aged 7-11 months, and the lowest was in the age group ≤ 28 d. The detection rate was the highest in spring and the lowest in autumn. The positive rate of β -lactamase was 92.0%, the resistance rate to ampicillin was 92.0%, the sensitivity to amoxicillin/clavulanate was 70.2%, and the sensitivity to cefotaxime, ofloxacin, tetracycline, chloramphenicol, and rifampicin was more than 90.0%.

Conclusions: Children aged 7 months to 14 years were generally susceptible to *H. influenzae* in spring, and the positive rate of β -lactamase was high. Doctors should refer to the infection characteristics and drug resistance of *H. influenzae* and choose antibiotics correctly to better control the infection.

Keywords: Children, *Haemophilus influenzae*, Antibiotic resistance, Chengdu, China

1. Background

Haemophilus influenzae is a Gram-negative bacillus that has a capsule and a high colonization rate in the respiratory tract and can cause infection in many parts of the body, including otitis media, epiglottitis, sinusitis, and pneumonia (1). Children are generally susceptible to bacteria because of their special physiological and immune status. The main route of transmission of *H. influenzae* is respiratory droplets, and a special route of transmission in children is through inhalation of maternal amniotic fluid or exposure to genital tract secretions during spontaneous delivery (2). Due to the strong pathogenicity of *H. influenzae*, if children are not actively treated after infection, it may lead to serious consequences, such as bacteremia and meningitis (3). With the overuse of antibiotics and the de-

velopment of various drug resistance mechanisms, the resistance rates of *H. influenzae* to ampicillin and first-line cephalosporins are rising (4, 5). In recent years, due to the irregular use of antibiotics in children, the drug resistance rate of *H. influenzae* has increased (6). Laboratory support is urgently needed for its treatment, including early identification of *H. influenzae* and selection of antibiotics it will be sensitive to (7).

2. Objectives

The aim of this study was to investigate *H. influenzae* infections in the lower respiratory tract of children in Chengdu and to evaluate the antibiotic resistance in the Chengdu area between February 2018 and January 2020, so

as to provide a reference for the clinical diagnosis of *H. influenzae* infection and the rational use of antibiotics.

3. Methods

3.1. Subjects

A total of 15891 clinical isolate strains in sputum were collected from outpatients and inpatients at Chengdu Women's and Children's Central Hospital, School of Medicine, University of Electronic Science and Technology of China. Patients from the Chengdu region were selected over a 2-year period from February 2018 to January 2020. The subjects were between 1 day and 14 years of age and included 9236 males and 6655 females. There were 1786 newborns (≤ 28 days), 5118 infants (29 days-6 months) and 2058 babies aged 7 months to 11 months; 6010 children were aged 1-3 years, 792 were aged 4 - 6 years, and 127 were aged 7 - 14 years. According to the season, the sputum samples were divided into four groups: spring, summer, autumn and winter, including 3987 cases in spring, 3267 cases in summer, 3266 cases in autumn and 5371 cases in winter.

3.2. Specimen Collection

According to the literature (8), specimens were collected by physicians and put into a sterile container. Then the specimens were sent to the laboratory, and proper screening of sputum with Gram staining was performed immediately. A total of 20 - 40 visual fields were detected under microscope, according to the qualified standard of sputum: sputum specimens containing > 25 leukocytes but < 10 squamous epithelial cells per low-power field (LPF, $\times 100$) were considered of good quality for bacterial culture in patients. Otherwise, the sputum sample was considered contaminated by saliva and rejected (9).

3.3. Identification of *Haemophilus influenzae*

Sputum specimens were cultured in accordance with the operational requirements of the fourth edition of the National Rules for Clinical Examination. The plates were placed at 35°C and cultured in a 5 - 10% CO_2 incubator for 18 - 24 hours. Clear or translucent, flat, moist, dew-drop colonies were selected, confirmed as Gram-negative bacilli under the microscope, and then identified using the VITEK 2 compact automatic microbial identification system (BioMerieux, France) (7).

3.4. Antimicrobial Susceptibility Testing

According to the Clinical and Laboratory Standards Institute (CLSI) disk diffusion methods, the minimum inhibitory concentrations (MICs) of ampicillin, amoxicillin-clavulanic acid, cefuroxime, cefaclor, cefotaxime, tetracycline, ofloxacin, chloramphenicol, trimethoprim-sulfamethoxazole, and rifampicin were determined by an ATB HAEMO drug sensitivity testing system (BioMerieux, France) using *Haemophilus* test medium (HTM) (Chongqing PangTong Medical Devices Co., China). The interpretative breakpoints were based on the CLSI criteria (<http://www.clsi.org/clinical-breakpoints/>). Testing was performed at 35°C for 18 - 24 hours. ATCC reference *H. influenzae* 49247 (Microbiologics, USA) was used for quality control. β -Lactamase production was revealed by nitrocefin sticks. The antibiotic susceptibility results were determined according to the CLSI 2020 standard and were analyzed by WHONET 5.6 statistical software (10, 11).

3.5. Statistical Analysis

SPSS Statistics version 22 (SPSS Inc. Chicago, IL, USA) was used to analyze the data. The statistical test used for all data was the chi-square test. $P < 0.05$ was considered to be statistically significant.

4. Results

4.1. Detection Rate of *Haemophilus influenzae*

A total of 5488 strains of *H. influenzae* were detected from 15891 clinical isolate strains in sputum collected from February 2018 to January 2020, for a positive rate of 34.54%.

4.2. Relationship Between *Haemophilus influenzae* Infection and Sex

Among the 5488 strains of *H. influenzae* detected from 15891 clinical isolate strains in sputum, there were 3197 males and 2291 females. Sex comparison showed that there was no significant difference ($P > 0.05$), as shown in Table 1.

4.3. Detection Rate of *Haemophilus influenzae* in Patients in Different Age Groups

Patients were divided into six groups: (1) ≤ 28 days old; (2) 29 days to 6 months old; (3) 7 to 11 months old; (4) 1 to 3 years old; (5) 4 to 6 years old; and (6) 7 to 14 years old. The results showed that the detection rates of *H. influenzae* in groups 3, 4, 5 and 6 were higher than those in groups 1 and 2. Group 3 had the highest detection rate, and group 1 had the lowest infection rate (Table 2).

Table 1. The Prevalence of *Haemophilus influenzae* in Sputum in Terms of Gender. ($\chi^2 = 0.058, P = 0.810$)

Gender	Number of specimens	Number of <i>Haemophilus influenzae</i>	Prevalence (%)
Male	9236	3197	34.61
Female	6655	2291	34.43
Total	15891	5488	34.54

Table 2. The Prevalence of *Haemophilus influenzae* in Sputum in Terms of Age Group. ($\chi^2 = 1006.618, P < 0.001$)

Age group	Number of Specimens	Number of <i>Haemophilus influenzae</i>	Prevalence (%)
≤ 28 d	1786	137	7.67
29 d - 6 months	5118	1438	28.10
7 - 11 months	2058	981	47.67
1 - 3 years	6010	2541	42.28
4 - 6 years	792	335	42.30
7 - 14 years	127	56	44.09
Total	15891	5488	34.54

4.4. Relationship Between *Haemophilus influenzae* Infection and Season

Among the 5488 strains of *H. influenzae* detected, the detection rate in children in summer vs. winter was not significantly different, but it was significantly different between spring and autumn. The prevalence of *H. influenzae* in sputum was the highest in spring and the lowest in autumn, as shown in Table 3.

4.5. Analysis of Antimicrobial Susceptibility Testing

Of the 5488 strains detected, 5049 (92.0%) were positive for β -lactamase. The resistance rate of the *H. influenzae* isolates to ampicillin was 92.0%. The resistance rates to cefuroxime, cefaclor and trimethoprim-sulfamethoxazole were all more than 60.0%. It was found that the sensitive rate of *H. influenzae* to tetracycline, chloramphenicol and rifampicin was more than 90.00%. The sensitive rate to amoxicillin/clavulanate was 70.2%. No resistance to cefotaxime or ofloxacin was detected (Table 4).

5. Discussion

Haemophilus influenzae is a common pathogen of community-acquired infection in children (12, 13). Bacteria mainly cause infection in children, including pneumonia, otitis media, cellulitis, arthritis, meningitis, bloodstream infection and other diseases, making it a serious threat to children's health (14, 15). In recent years, with the overuse of antibiotics in children, the drug-resistant strains of *H. influenzae* are growing in number, which brings great difficulties to treatment (7).

Owing to the imperfect development of children's respiratory system, relatively low immune function and the high microbial colonization rate, the infection rate of *H. influenzae* in children has been very high (16, 17). For economic reasons and lack of awareness of *H. influenzae*, although the Chinese government recommended that infants be vaccinated against *H. influenzae* nationwide and launched a self-pay *H. influenzae* type b strain vaccine, there are still many unvaccinated children, and the infection caused by non-type b or non-typeable *H. influenzae* strains is still a serious problem. These strains are currently the most common cause of invasive *H. influenzae* infection in many countries (18, 19).

In this study, we conducted a retrospective investigation to study the epidemic characteristics and antibiotic resistance of *H. influenzae* in children aged 0 - 14 years with lower respiratory tract infection in Chengdu, China. Our results showed that 5488 strains of *H. influenzae* were detected, and the total positive rate of *H. influenzae* in this study was 34.54%. This indicated that *H. influenzae* infection was high in children with lower respiratory tract infections in the Chengdu region. This result supports one previous investigation that suggested that *H. influenzae* was one of the most common causes of community-acquired pneumonia in children (20).

In our study, sex comparison showed that there was no significant difference. This result indicates that there was no sex difference in *H. influenzae* infection in children in Chengdu. The age distribution of *H. influenzae* infection in our hospital was most concentrated from 29 days to 14 years old, as the detection rates of *H. influenzae* in age groups 3, 4, 5 and 6 were significantly higher than those

Table 3. The Prevalence of *Haemophilus influenzae* in Sputum in Terms of Seasons. ($\chi^2 = 836.641$, $P < 0.001$)

Seasons	Number of Specimens	Number of <i>Haemophilus influenzae</i>	Prevalence (%)
Spring	3987	2020	50.67
Summer	3267	1079	33.03
Autumn	3266	605	18.52
Winter	5371	1784	33.22
Total	15891	5488	34.54

Table 4. Susceptibility of *Haemophilus influenzae* Strains to Antimicrobial Agents^a

Antibiotic	n	S	I	R
Ampicillin	5488	439 (8.0)	0 (0.0)	5049 (92.0)
Amoxicillin/clavulanate	5488	3853 (70.2)	0 (0.0)	1635 (29.8)
Cefuroxime	5488	1613 (29.4)	209 (3.8)	3666 (66.8)
Cefaclor	5488	1164 (21.2)	263 (4.8)	4061 (74.0)
Cefotaxime	5488	5488 (100.0)	0 (0.0)	0 (0.0)
Tetracycline	5488	5241 (95.5)	11 (0.2)	236 (4.3)
Ofloxacin	5488	5488 (100.0)	0 (0.0)	0 (0.0)
Chloramphenicol	5488	5263 (95.9)	5 (0.1)	220 (4.0)
Trimethoprim-sulfamethoxazole	5488	1690 (30.8)	0 (0.0)	3798 (69.2)
Rifampicin	5488	5483 (99.9)	0 (0.0)	5 (0.1)

Abbreviations: S, susceptible; I, intermediate; R, resistant.

^aValues are expressed as No. (%).

in age groups 1 and 2 ($P < 0.05$). Our study also showed that the detection rates of *H. influenzae* in the 7 - 11-month age group were the highest, and newborns under 28 days had the lowest infection rate. The reason for this result may be related to their pulmonary function and immunity. Since most of the patients came from the community, children less than 6 months old had maternal antibodies and higher resistance to community-acquired pneumonia. Several studies have shown that *H. influenzae* infection shows an obvious seasonal distribution, usually occurring in winter and spring, or between February and May (7, 20). Our study showed that the detection rates of *H. influenzae* in sputum were the highest in spring and the lowest in autumn, which was not consistent with the previous studies. This indicated that the seasonal distribution of *H. influenzae* infection may be different due to changes in climatic conditions.

With the development of various drug resistance mechanisms, such as β -lactamase activity and modified bacterial penicillin binding protein (21), the range of antibiotics active against *H. influenzae* has gotten narrower, especially given the limited set of antibiotics that can be given to children. It is of great significance to study the

antibiotic resistance of *H. influenzae* in children. Most *H. influenzae* strains are phenotypically β -lactamase negative and ampicillin susceptible (BLNAS), so ampicillin and amoxicillin/clavulanate, as well as third-generation cephalosporins such as cefotaxime, are among the drugs of choice for the treatment of *H. influenzae* infections (22), but in our study, of the 5488 strains detected, 5049 (92.0%) were positive for β -lactamase. The *H. influenzae* ampicillin resistance rate was 92.0%, which was higher than what had been found in previous studies in China (58.1%) (7), Iran (54.8%), Germany (11.6%) and Poland (29.1%) (7, 23), but close to the ampicillin resistance rate in South Korea (69.4%) (7).

These data suggested that the overall enzyme production rate of *H. influenzae* was high and had an increasing trend, which was also the main reason for the drug resistance of *H. influenzae*. The differences in the above results may also be due to differences in geographical location or the use of antibiotics by children. The sensitive rate to amoxicillin/clavulanate was 70.2%. Therefore, single penicillin-type drugs, such as ampicillin and amoxicillin, are not recommended in clinical treatment, but β -lactamase inhibitors are recommended for antibacterial treatment. The resistance rate of cefotaxime was very low,

which was consistent with previous studies in Taiwan and Germany (3, 22). The resistance rates to cefuroxime, cefaclor and trimethoprim-sulfamethoxazole were all more than 60.0%, and the sensitive rates of *H. influenzae* to tetracycline, chloramphenicol and rifampicin were more than 90.0%. Due to the limitations of their use in children, we do not recommend ofloxacin, tetracycline, and chloramphenicol for children. Since *H. influenzae* showed low resistance rates to amoxicillin/clavulanate and cefotaxime, they have good antibacterial activity in children with *H. influenzae* infection and might be appropriate for this population.

In recent years, the resistance rate of *H. influenzae* to ampicillin has gradually increased. Microbiological laboratories should pay attention to and strengthen the culture and drug resistance surveillance of *H. influenzae*. At the same time, physicians should choose antibiotics reasonably according to the results of drug sensitivity tests, control the spread and prevalence of drug-resistant bacteria, and avoid unreasonable drug use.

5.1. Conclusions

In conclusion, children aged 7 months to 14 years were generally susceptible to *H. influenzae* in spring, and the positive rate of β -lactamase reached 92.0%. Amoxicillin/clavulanate or cefotaxime can be used as the first choice for treatment. To better control the infection, physicians can refer to these characteristics and antibiotic resistance of *H. influenzae* for diagnosis and treatment.

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Footnotes

Authors' Contribution: Qin Wang conducted the specimen studies and drafted the manuscript; Cheng-Gui Liu contributed to the study design; Jian Xu, Qin Zhang and Pei-Pei Song participated in the susceptibility testing; Chong-Hui Zhao contributed to the statistical analysis.

Conflict of Interests: There were no conflicts of interest for any of the authors.

Ethical Approval: This study was done in accordance with the Helsinki Declaration and was approved by the Medical Ethics Committee at Chengdu Women's and Children's Central Hospital, Chengdu, China ((2013)2, Medical Ethics Committee, CWCCH). Consent from each participant was

waived because the present study was retrospective. Because all patient data were analyzed anonymously, no additional informed consent was required.

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