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Original Article

A retrospectively study on the detection rate and drug resistance of *Haemophilus influenzae* in children with respiratory tract infections in Nanjing and Wuhan

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Abstract: Objective: A retrospective study was carried out to explore the detection rate and drug resistance of *Haemophilus influenzae* (*H. influenzae*) in children with respiratory tract infections in Nanjing and Wuhan, so as to provide guidance for clinicians in rational drug use. Methods: The fresh sputum of 1,000 children with acute respiratory infections (ARI) hospitalized in Nanjing and Wuhan from January 2017 to December 2018 were collected using aseptic negative pressure suction, and *H. influenzae* was cultured and isolated from the samples. The detection rate and drug resistance to antibiotics of *H. influenzae* were retrospectively studied. Results: In Nanjing and Wuhan, *H. influenzae* infections occur mostly in children younger than 3 years old, and *H. influenzae* was detected in the sputum samples of more than 95.00% of these children. The detection rate of *H. influenzae* was high in winter and spring, reaching 56.80% in winter. The sensitivity of *H. influenzae* to ampicillin, amoxicillin, and cefuroxime decreased significantly in 2018 compared to 2017, while its sensitivity to β-lactamase and the compound sulfamethoxazole increased (all P≤0.001). Conclusion: *H. influenzae* infections in the respiratory tract are seasonal and occur mostly in winter. Antibiotics should be used reasonably according to the drug-susceptibility testing (DST) results in the clinic to avoid drug resistance.

Keywords: Respiratory tract infections in children, *Haemophilus influenzae*, detection rate, drug resistance

Introduction

Haemophilus influenzae (*H. influenzae*) is mainly parasitic in children's respiratory tracts, and it is not only the most predominant and common pathogen causing bacterial pneumonia in children, but also one of the important pathogens causing community-acquired pneumonia. It is reported that about 3 million children worldwide are infected with *H. influenzae* every year, and nearly 400,000 to 700,000 die because of it [1, 2]. In recent years, there has been an increasing number of studies on *H. influenzae* due to the abuse of antibiotics in China. Researchers have found that the β-lactamase-producing strains of *H. influenzae* are increasing along with diverse drug resistance mechanisms. Currently, the most commonly used antibiotics in clinics include azithromycin, amoxicillin, levofloxacin, and cefixime. However, studies have reported that, over time, *H. influenzae* will develop different degrees of resistance to these antibiotics. Therefore, the early analysis of the drug resistance of *H. influenzae* is of great significance for improving the clinical medication and prognoses of children [3, 4]. In view of this, 1,000 children with respiratory tract infections admitted to Renmin Hospital of Wuhan University from January 2017 to December 2018 were enrolled in this study.

Data and methods

Baseline data

The study was conducted from January 2017 to December 2018 and was approved by the Ethics Committee of Renmin Hospital of Wuhan University. A total of 1,000 children with acute respiratory infections (ARI) (500 in Nanjing and 500 in Wuhan) admitted to Renmin Hospital of

Table 1. The age distribution of *H. influenzae* cases in Nanjing and Wuhan

Age		Number of cases (n)	Percentage (%)
Nanjing	≤3 years old	485	97.00
	>3 years old	15	3.00
Wuhan	≤3 years old	480	96.00
	>3 years old	20	4.00

Table 2. The distribution of *H. influenzae* specimens in Nanjing and Wuhan

	Bronchoalveolar lavage specimens	Sputum
Nanjing (n, %)	20 (4.00)	480 (96.00)
Wuhan	22 (4.40)	478 (95.60)
X^2	0.099	
P value	0.753	

Wuhan University during the period were enrolled in our study, with the ratio of female to male of 505:495, an average age of (7.52± 2.05) years, weighing (9.6-18.9) kg, and an average weight of (14.06±2.44) kg.

Isolation and identification, and drug-susceptibility testing (DST)

The children's sputum was collected using aseptic negative pressure suction. Bronchoalveolar lavage (BAL): Doubtful lesion regions on the bronchus were repeatedly flushed with physiological saline, then the bronchoalveolar lavage fluid (BALF) was collected using negative pressure suction. Afterwards, the BALF was centrifuged at 2,000 r/min for 3 min, the supernatant was discarded, and the sediment was mixed evenly for a smear examination. The Kirby-Bauer disc diffusion (K-B) method and the dilution method were used for the DST. The sensitivity of *H. influenzae* to azithromycin, ampicillin, amoxicillin, chloramphenicol, ertapenem, levofloxacin, compound sulfamethoxazole, cefixime and other antibiotics was determined using "ATB HAEMO" test strips. *H. influenzae* was placed into suspension in distilled water or saline, then transferred to a growth medium and inoculated on an "ATB HAEMO" test strip for 18-24 hours, and the results were observed [5, 6].

K-B disc method: Pure culture colonies with a similar morphology selected from agar plate were inoculated in a broth medium and cultured at 35°C until the turbidity ≥0.5 the McFarland standard. The colonies were taken out, and the bacterial concentration was adjusted to 0.5 McFarland standard with the broth, with a bacteria content of (1-2) $\times 10^8$ cfu/mL. Sterile cotton swabs were impregnated into the adjusted bacterial suspension, and the excess suspension was extruded. The swabs were used to makes streaks on the entire surfaces of the Mueller-Hinton (M-H) agar plates, the plates were rotated to 60° and the streaking was repeated three times, and the edges of the agar were smeared one last time. Afterwards, the plates were placed at room temperature for 3-5 min. Then a disc dispenser or a pair of sterile tweezers was used to make the paper discs stick to the surfaces of the plates, and the discs were gently pressed with the tip of the tweezers to ensure full contact. After the plates were incubated at 35° for 18-24 hours, the diameters of the inhibition zones were measured using a Vernier caliper [7, 8].

Statistical analysis

SPSS 17.0 (Chicago, IL, USA) was used for the data collation, calculation and analysis. The measurement data were expressed as the means ± standard deviations ($\bar{x}$ ± sd), and the counting data were expressed as n (%). The comparisons between groups were conducted using X^2 tests. A value of $P<0.05$ was considered statistically significant.

Results

Age distribution of H. influenzae

Most of the children with H. influenzae infections in Nanjing and Wuhan were younger than 3 years old, as shown in **Table 1**.

Distribution of H. influenzae specimens

The distribution of the *H. influenzae* specimens in Nanjing and Wuhan are shown in **Table 2**. *H. influenzae* was detected in the sputum samples of more than 95.00% of the children.

Detection rate of H. influenzae in different seasons

In winter (December-February), the detection rate of H. influenzae in Nanjing and Wuhan was the highest (both P=0.001), reaching 56.80% (**Table 3** and **Figure 1**).

Table 3. The detection rate of *H. influenzae* in different seasons

	Spring (March-May, %)	Summer (June-August, %)	Autumn (September-November, %)	Winter (December-February, %)	X^2	*P* value
Total	20.50	9.20	13.50	56.80		
Nanjing	20.80	15.60	17.20	52.40	288.030	0.001
Wuhan	29.20	9.80	18.60	66.80	440.630	0.001
X^2	9.408	7.585	0.333	21.531		
P value	0.002	0.006	0.564	0.001		

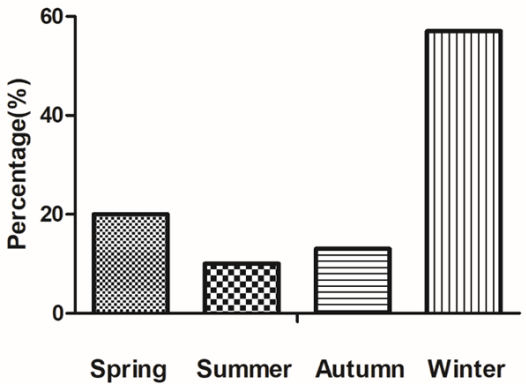

Figure 1. Total detection rate of H. influenzae in different seasons.

The sensitivity of H. influenzae to antibiotics in different years

From 2017 to 2018, the sensitivity of *H. influenzae* to ampicillin, amoxicillin and cefuroxime decreased from 62.50%, 100%, and 100% to 40.50%, 96.00%, and 92.00% respectively (all P<0.001). In addition, the positive percentages of β-lactamase and the compound sulfamethoxazole increased from 11.60% and 51.60% to 42.80% and 58.80% respectively (P≤0.001). The sensitivity of *H. influenzae* to antibiotics in 2017 and 2018 is shown in **Table 4**.

Discussion

H. influenzae, an opportunistic pathogen, is mostly parasitic on the human upper respiratory tract and was first extracted from the nasopharynx by Polish bacteriologist Pfeiffer in 1892. *Haemophilus* is common in people infected with upper respiratory tract viruses, of which *Haemophilus* type B (HiB) is the most invasive, and it is mainly transmitted through the air and droplets. *H. influenzae* infection in children is usually secondary to infectious diseases such as measles, whooping cough, tuberculosis, and influenza, with the specific manifestations of shortness of breath, expectoration, cough, and fever. It is an important cause of respiratory tract infections in children in China, mainly occurring in children under 5 years old. Studies have found that the probability of children being infected with *Haemophilus* reaches 20%, and the infection usually causes cellulitis, otitis media, septicemia, arthritis, and other infectious diseases [8-10]. Patients with immune dysfunction or exogenous colds are prone to pneumonia and upper respiratory tract infections [11]. Our study showed that the infection rate of H. influenzae was about 96.00% in children under 3 years old. Due to their imperfect development of the organs and tissues, poor lung function, as well as low immune resistance, children are very vulnerable to pathogenic bacteria, which has a serious adverse impact on their body and intellectual growth [12, 13]. The abuse of antibiotics in China has led to a significant increase in drug-resistant strains. In the 1970s ampicillin replaced tetracycline and chloramphenicol and became the main antibiotic for the treatment of *Haemophilus*. Therefore, increasing the detection rate of *H. influenzae* and reducing its drug resistance play important roles in children's health.

H. influenzae is an aerobe or facultative anaerobe with high nutritional requirements, whose growth requires the participation of X and V factors. It is precisely because of the high requirements of the culture methods and conditions and the influence of the specimen quality and the sampling site that the detection rate of H. influenzae is generally low. Therefore, an appropriate medium and CO_2 concentration are necessary for *H. influenzae* culture. Previous studies reported that the isolation rate of *H. influenzae* in a rabbit blood chocolate agar medium containing vancomycin (50 μg/mL) was significantly higher than it was in other media [6, 7, 14-16]. While vancomycin has an inhibitory effect on gram-negative bacteria, the colonies

Table 4. The sensitivity of *H. influenzae* in different years (n, %)

Antibiotics	2017		2018		*P* value
	Number of cases (n)	Percentage (%)	Number of cases (n)	Percentage (%)	
β-lactamase positive	116	11.60	428	42.80	<0.001
azithromycin	1,000	100.00	1,000	100.00	
ampicillin	625	62.50	405	40.50	<0.001
amoxicillin	1,000	100.00	960	96.00	<0.001
chloramphenicol	1,000	100.00	1,000	100.00	
utapenem	1,000	100.00	1,000	100.00	
levofloxacin	1,000	100.00	1,000	100.00	
compound sulfamethoxazole	516	51.60	588	58.80	0.001
cefuroxime	1,000	100.00	920	92.00	<0.001

of *H. influenzae* will grow rapidly and be easily identified, which greatly improves its detection rate [12, 17-20]. Therefore, in this study, a rabbit blood chocolate agar medium containing 50 μg/mL vancomycin was employed to culture *H. influenzae*. In addition, we found that the isolation rate of *H. influenzae* in the throat was significantly higher than it is in the saliva, so the specimens of *H. influenzae* were collected from the children's throat swabs in this study.

Our study found that there is a notable seasonal correlation in the detection rate of *H. influenzae*. The detection rate reached 56.80% in the winter (December to February), significantly higher than that in other three seasons, which is consistent with the previous research results. This is mainly because the respiratory mucosal functions and ciliary movements, which are significantly weakened in winter, prevent secretions from being discharged normally and reduce the patency of the respiratory tract, thereby increasing the survival rate of H. influenzae. In addition, this study suggested that the sensitivity of *H. influenzae* to amoxicillin and cefuroxime decreased significantly from 2017 to 2018, indicating that the drug resistance of H. influenzae is still increasing. Therefore, clinicians need to strengthen their monitoring to effectively improve the detection rate of *H. influenzae* and standardize and rationally use antibiotics to eliminate their abuse [13, 21, 22]. Moreover, hospitals and communities should enhance their universal education on *H. influenzae* infection to minimize children's infection.

To sum up, the infection rate of *H. influenzae* is relatively high in winter (December to February). Clinically, patients should be guided to increase their exercising in winter to enhance their physical fitness and improve their immune function and resistance to prevent *H. influenzae* infection. At the same time, DST should be performed more frequently to select economical, reasonable, effective and safe antibiotic treatments and to minimize adverse reactions and drug resistance.

Disclosure of conflict of interest

None.

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