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Haemophilus influenzae Vulvovaginitis in Prepubertal Girls: A 4-Year Study on a Tertiary Children's Hospital in China

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Abstract

Background: Vulvovaginitis is a common infection in prepubertal girls, which is partly caused by bacterial infection. According to the literature, *Haemophilus influenzae* is one of the most common bacterial causes of vulvovaginitis in children. However, few studies with large sample sizes have delved into this issue.
Objectives: To determine the prevalence of *Haemophilus influenzae* vulvovaginitis in prepubertal girls and detect the antimicrobial resistance of *H. influenzae* strains isolated from vulval specimens.
Methods: The isolates of *H. influenzae* from the vulval swabs of prepubertal girls with vulvovaginitis were received from The Children's Hospital, Zhejiang University School of Medicine, during 2016 - 2019. The vulval specimens were inoculated on *Haemophilus* selective chocolate agar, and antimicrobial susceptibility tests were performed by the disk diffusion method. Moreover, β-lactamase was detected using Cefinase disc.
Results: In this study, 4142 vulval specimens were received during four years, of which 649 *H. influenzae* isolates had been isolated from 642 girls aged 6 months-13 years, with a median of 5 years. The peaks of isolates were observed from April to July in the vulval isolates. In general, the ampicillin resistance rate was 39.1% (250/640), 33.2% of strains (211/636) were β-lactamase-positive isolates, and 6.6% strains (42/635) were β-lactamase-negative ampicillin-resistant (BLNAR) isolates. The resistance rates of *H. influenzae* isolates to amoxycillin-clavulanic acid, ampicillin-sulbactam, cefuroxime, ceftriaxone, cefotaxime, meropenem, levofloxacin, sulfamethoxazole-trimethoprim, azithromycin, and chloramphenicol were 26.4%, 21.8%, 24.8%, 1.7%, 1.0%, 0.2%, 0%, 47.7%, 10.2%, and 1.1%, respectively. Multi-drug resistance (MDR) was noticed in 41 persons (6.4%) out of the 642 *H. influenzae* isolates, with the most prevalent MDR phenotype of ampicillin-sulfamethoxazole-trimethoprim-azithromycin resistance.
Conclusions: Clinicians should noticed that *H. influenzae* is a common bacterial cause of vulvovaginitis in children, and laboratories should routinely cover *Haemophilus* culture media for vulval specimens. The ampicillin resistance of *H. influenzae* should also be considered in clinical management.

Keywords: *Haemophilus influenzae*, Vulvovaginitis, Prepubertal, Antimicrobial Resistance, Children

1. Background

Vulvovaginitis is a common problem in prepubertal girls, which is mainly the result of poor hygiene or nonspecific irritants as well as bacterial infection (1). *Streptococcus pyogenes* and *Haemophilus influenzae* (*H. influenzae*) are reported as the most common bacterial cause of vulvovaginitis in prepubertal girls (1-4). In 1987, MacFarlane first highlighted the relationship between *H. influenzae* and prepubertal vulvovaginitis (5). Cox's (4) study suggested that *H. influenzae* was an underrated cause of vulvovaginitis among young girls. However, few studies have comprehensively explored the prevalence of *H. influenzae* vulvovaginitis in prepubertal girls. Furthermore, few studies with large sample sizes have been conducted in this regard.

2. Objectives

Accordingly, we described a four-year study from a tertiary university children's hospital to determine the prevalence of *H. influenzae* vulvovaginitis in prepubertal girls and detect the antimicrobial resistance of *H. influenzae* strains isolated from vulval specimens.

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3. Methods

3.1. Strain Collection and Identification

This retrospective analysis examined the data collected from prepubertal girls referred to the outpatient clinic of pediatric and adolescent gynecology at The Children's Hospital, Zhejiang University School of Medicine, from January 2016 to December 2019. One vulval swab was taken for each for microscopic examination. Moreover, another vulval swab was received for culture. To this end, Columbia blood agar, Chocolate agar (i.e., *Haemophilus* and Gonorrhoeae selective chocolate agar), and Sabouraud's agar were used. The suspected pathogens on *Haemophilus* selective chocolate agar were identified using the Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS, Bruker).

3.2. β-Lactamase Detection and Antimicrobial Susceptibility Test

The antimicrobial susceptibility test was performed using disk diffusion, and the results were interpreted according to the Clinical Laboratory Standards Institute's (CLSI) guidelines M100-S29. The resistance rates of *H. influenzae* isolates to ampicillin, amoxycillin-clavulanic acid, ampicillin-sulbactam, cefuroxime, ceftriaxone, cefotaxime, meropenem, levofloxacin, sulfamethoxazole-trimethoprim, azithromycin, and chloramphenicol (Oxoid, UK) were also detected. In this regard, *H. influenzae* ATCC49247 was used as a quality control strain, β-lactamase was detected by Cefinase disc (BioMérieux, France), and *H. influenzae* isolates resistant to three or more different types of antibiotics were defined as Multi-Drug Resistance (MDR) isolates.

3.3. Statistical Analysis

The antibiotic-resistant rates were analyzed with WHONET software version 5.6. The antibiotic-resistant rates between different groups were also compared and analyzed using the chi-square test. Medians (IQR) were used to describe age data, and $P < 0.05$ was considered as the significance level.

4. Results

4.1. Isolates and Distribution

From January 2016 to December 2019, 4142 vulval swabs were received from prepubertal girls with vulvovaginitis, and 649 swabs (15.7%) were obtained from 642 patients with *H. influenzae*. The number of specimens received was increased from 803 in 2016, 931 in 2017, 920 in 2018 to 1488 in 2019; however, the proportion of *H. influenzae* positives was decreased from 18.6% (149/803) in 2016, 15.5% (144/931) in 2017, 16.8% (155/920) in 2018, to 13.0% (194/1488) in 2019. The peaks were noticed from April to July in the vulval isolates positive for isolates of *H. influenzae*. The age of the children with the *H. influenzae* isolates ranged from 0.5 to 13 years; however, 477 persons (75%) were in the age range of 3 - 7 years, with a median of 5 years (IQR: 3).

4.2. β-Lactamase Detection and Antimicrobial Susceptibility Test

In this study, the ampicillin resistance rate was 39.1% (250/640), of which 33.2% of the strains (211/636) were for β-lactamase-positive isolates, and 6.6% of the strains (42/635) were β-lactamase-negative ampicillin-resistant (BLNAR) isolates. The resistance rates of *H. influenzae* isolates to amoxycillin-clavulanic acid, ampicillin-sulbactam, cefuroxime, ceftriaxone, cefotaxime, meropenem, levofloxacin, sulfamethoxazole-trimethoprim, azithromycin, and chloramphenicol were 26.4%, 21.8%, 24.8%, 1.7%, 1.0%, 0.2%, 0%, 47.7%, 10.2%, and 1.1%, respectively (Table 1). The resistance rates of *H. influenzae* strains to cefuroxime and azithromycin revealed significant statistical differences over different years ($P < 0.05$; Table 2). β-lactamase-positive *H. influenzae* strains showed significantly higher resistance to ampicillin, amoxycillin-clavulanic acid, cefuroxime, sulfamethoxazole-trimethoprim, azithromycin, and chloramphenicol, compared to β-lactamase-negative strains ($P < 0.01$; Table 3). BLNAR *H. influenzae* strains were all resistant to amoxycillin-clavulanic acid, ampicillin-sulbactam, and cefuroxime; however, they were susceptible to levofloxacin, azithromycin, and chloramphenicol (Table 4).

4.3. MDR Pattern

Of the 642 *H. influenzae* isolates, MDR was present in 41 cases (6.4%). Ampicillin-sulfamethoxazole-trimethoprim-azithromycin resistance was the most prevalent resistance phenotype, which was detected in 16 isolates, representing 39% of the MDR strains (Table 5).

5. Discussion

Vulvovaginitis in prepubertal children is a common infection in clinical practice. Given the anatomy of the vulva at prepubertal age, it is vulnerable to infection in prepubertal children (1). Few hospitals can provide pediatric and gynecological outpatient services; hence, children with vulvovaginitis mainly receive the primary care

Table 1. Antibiotic Resistances of *H. influenzae* Strains Isolated from Vulval Specimens, 2016 - 2019[a]

Antibiotic	N	R, %	I, %	S, %
β-lactamase	636	33.2		66.8
Ampicillin	640	39.1	6.6	54.4
Amoxycillin-clavulanic acid	349	26.4	0	73.6
Ampicillin-sulbactam	641	21.8	0	78.2
Cefuroxime	640	24.8	2.5	72.7
Ceftriaxone	350	1.7*	0	98.3
Cefotaxime	288	1*	0	99
Meropenem	632	0.2*	0	99.8
Levofloxacin	640	0*	0	100
Sulfamethoxazole-trimethoprim	641	47.7	1.4	50.9
Azithromycin	469	10.2*	0	89.8
Chloramphenicol	641	1.1	0	98.9

Abbreviations: N, number of isolates; R, resistant; I, intermediate; S, susceptible.
[a] *, Rate of non-susceptible cases.

Table 2. Antibiotic Resistances of *H. influenzae* Strains Isolated from Vulval Specimens Over Different Years[a]

Antibiotic	2016		2017		2018		2019		P-Value
	N	R, %	N	R, %	N	R, %	N	R, %	
β-lactamase	148	31.8	142	26.8	156	35.9	192	37	0.209
Ampicillin	148	33.1	145	37.2	155	41.9	194	42.8	0.254
Amoxycillin-clavulanic acid	-	-	-	-	154	23.4	194	28.9	0.249
Ampicillin-sulbactam	149	16.1	145	26.9	155	21.3	194	22.7	0.161
Cefuroxime	149	17.4	145	26.2	154	23.4	194	30.4	0.048
Ceftriaxone	-	-	-	-	155	1.3*	194	2.1*	0.582
Cefotaxime	146	1.4*	143	0.7*	-	-	-	-	0.574
Meropenem	148	0*	137	0.7*	155	0*	194	0*	0.304
Levofloxacin	148	0*	145	0*	155	0*	194	0*	-
Sulfamethoxazole-trimethoprim	149	49	145	45.5	155	47.7	194	48.5	0.936
Azithromycin	-	-	-	-	155	7.1*	191	14.7*	0.027
Chloramphenicol	149	1.3	145	1.4	155	0	194	1.5	0.515

Abbreviations: N, number of isolates; R, resistant.
[a] *, Rate of non-susceptible cases.

(3). In this regard, few studies have comprehensively explored the prevalence of *H. influenzae* vulvovaginitis in prepubertal girls. To this end, the present study was performed at a tertiary university hospital providing specialist pediatric, and gynecological outpatient services.

Vulvovaginitis is one of the most common gynecological problems among prepubertal girls. In this regard, a multicenter study showed the leading cause of pediatric inflammatory vulvovaginitis to be the upper respiratory tract pathogens (6). A case report first documented the nose-hand-vagina method of transmission for vulvovaginitis (7), which assumed that respiratory pathogens were transmitted to the vulvar area via the hands (8). Accordingly, hand hygiene and behaviors would be an essential strategy to prevent vulvovaginitis in prepubertal girls.

Several studies have indicated that vulvovaginitis in prepubertal girls is mainly caused by the bacteria from the upper respiratory tract, *S. pyogenes*, and *H. influenzae* (1). *H. influenzae* more commonly caused vulvovaginitis than β *haemolytic streptococci* in Liverpool (9). However, *H. influenzae* is fastidious for growth requirements; hence, laboratories should not isolate it unless they cover the appropriate culture medium of *Haemophilus* for vulval swabs (10). In the present study, all the specimens were inoculated on the selective chocolate agar of *Haemophilus* to isolate *H. influenzae*.

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Table 3. Comparng Antibiotic Resistance Between β-Lactamase-Positive and β-Lactamase-Negative *H. influenzae* Strains Isolated from Vulval Specimens, 2016 - 2019[a]

Antibiotic	β-Lactamase (+)				β-Lactamase (-)				P-Value
	N	R, %	I, %	S, %	N	R, %	I, %	S, %	
Ampicillin	211	98.6	0.5	0.9	424	9.9	9.4	80.7	0.000
Amoxycillin-clavulanic acid	126	38.9	0	61.1	222	18.9	0	81.1	0.000
Ampicillin-sulbactam	212	26.9	0	73.1	424	19.6	0	80.4	0.046
Cefuroxime	212	33	6.6	60.4	423	21	0.5	78.5	0.001
Ceftriaxone	127	3.1*	0	96.9	222	0.9*	0	99.1	0.272
Cefotaxime	83	1.2*	0	98.8	201	1*	0	99	0.626
Meropenem	210	0*	0	100	417	0.2*	0	99.8	0.608
Levofloxacin	211	0*	0	100	424	0*	0	100	/
Sulfamethoxazole-trimethoprim	212	59.4	0.5	40.1	424	42	1.9	56.1	0.000
Azithromycin	161	28*	0	72	307	1*	0	99	0.000
Chloramphenicol	212	3.3	0	96.7	424	0	0	100	0.001

Abbreviations: N, number of isolates; R, resistant; I, intermediate; S, susceptible.
[a] *, Rate of non-susceptible cases.

Table 4. Antibiotic Resistances of BLNAR *H. influenzae* Strains Isolated from Vulval Specimens, 2016 - 2019[a]

Antibiotics	N	R, %	I, %	S, %
Amoxycillin-clavulanic acid	22	100	0	0
Ampicillin-sulbactam	42	100	0	0
Cefuroxime	42	100	0	0
Ceftriaxone	22	9.1*	0	90.9
Cefotaxime	20	10*	0	90
Meropenem	36	2.8*	0	97.2
Levofloxacin	42	0*	0	100
Sulfamethoxazole-trimethoprim	42	47.6	9.5	42.9
Azithromycin	24	0*	0	100
Chloramphenicol	42	0	0	100

Abbreviations: N, number of isolates; R, resistant; I, intermediate; S, susceptible.
[a] *, Rate of non-susceptible cases.

Previous studies have described a variety of bacteria as the possible causes of vulvovaginitis in children. However, signs of inflammation associated with pure or predominant growth may be diagnostic relevance of pathogenic microorganisms (1). In this study, the large number of polymorphonuclear leukocytes in the microscopic examination revealed the inflammatory reaction, implying that the *H. influenzae* isolated from the vulval swabs was a possible pathogenic microorganism. In this study, of 4142 vulval swabs, 649 swabs (15.7%) were from children with *H. influenzae*. This issue was in agreement with the opinions described above, suggesting that *H. influenzae* was a common pathogen of vulvovaginitis in children in Zhejiang, China. The peaks of isolates were noticed during April-July in the vulval isolates, which was consistent with the peaks of respiratory tract specimens, suggesting that vulval *H. influenzae* strains might be transmitted from the respiratory tract (6, 11). The age of children with *H. influenzae* ranged from 0.5 to 13 years; however, 477 children (75%) were aged between 3-7 years. This finding was in line with those of the previous studies (11, 12).

Since the 1970s, ampicillin was used as an option to treat *H. influenzae* infections (13). In recent years, because of the extensive use of antibiotics, the drug resistance of *H. influenzae* to ampicillin has gradually increased. The ampicillin resistance rate of *H. influenzae* strains in China was increased from 12% during 2000 - 2002 (14) to 58.1% in 2016 (15). In this study, the ampicillin resistance rate was 39.1%, which was higher than the rate in genital strains (26.4%) and lower than that in respiratory strains (58.4%) in 2015, as reported by our research team (15). The ampicillin resistance of *H. influenzae* strains isolated from vulval specimens gradually increased from 33.1% in 2016 to 42.8% in 2018; hence, the ampicillin resistance of *H. in-*

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Table 5. Main MDR Patterns of *H. influenzae* Strains Isolated from Vulval Specimens, 2016 - 2019

MDR pattern	No. (%)
β-lactams-SXT-AZM	
AMP-SXT-AZM	16 (39.0)
AMP-CXM-SXT-AZM	4 (9.8)
AMP-CXM-AMC-SXT-AZM	3 (7.3)
AMP-CXM-SAM-AMC-SXT-AZM	3 (7.3)
AMP-AMC-SXT-AZM	2 (4.9)
AMC-SXT-AZM	1 (2.4)
AMP-CXM-SAM-SXT-AZM	1 (2.4)
SAM-SXT-AZM	1 (2.4)
CXM-SXT-AZM	1 (2.4)
AMP-SAM-AMC-SXT-AZM	1 (2.4)
AMP-CXM-SAM-AMC-CRO-SXT-AZM	1 (2.4)
β-lactams-SXT-CHL	
AMP-SXT-CHL	3 (7.3)
AMP-AMC-SXT-CHL	1 (2.4)
AMP-SAM-SXT-CHL	1 (2.4)
AMP-CXM-SXT-CHL	1 (2.4)
β-lactams-SXT-AZM-CHL	
AMP-SXT-AZM-CHL	1 (2.4)

Abbreviations: AMP, ampicillin; AMC: amoxycillin-clavulanic acid; AZM: azithromycin; CHL: chloramphenicol; CRO: ceftriaxone; CXM: cefuroxime; SAM: ampicillin-sulbactam; SXT: sulfamethoxazole-trimethoprim.

fluenzae should be considered in clinical management. In the present study, the main mechanism of ampicillin resistance in *H. influenzae* isolates was the production of β-lactamase. This finding was in agreement with those in some other studies (12, 16, 17) and in contrast with those reported in Japan. Regarding the inconsistency of the findings, BLNAR accounted for more than 50% of cases after 2014 (18) and only 6.6% in this study, suggesting significant differences among different countries regarding the antibiotic resistance and mechanisms of *H. influenzae* isolates. Some studies have also compared *H. influenzae* resistance profiles between the respiratory tract and urinary tract (19), respiratory isolates and vaginal isolates (11), suggesting that the resistance profiles of *H. influenzae* vary greatly depending on the infection site. This finding also indicates that the optimal antibiotic treatment for *H. influenzae* may differ depending on the infection region and infection site. The resistance rates of the *H. influenzae* isolates to amoxycillin-clavulanic acid, and ampicillin-sulbactam were 26.4%, 21.8% in this study, which might be attributed to the BLNAR strains and the β-lactamase-producing clavulanic acid/amoxicillin-resistant (BLPACR) strains of *H. influenzae*. Furthermore, β-lactamase and PBP amino acid substitutions might be the mechanisms of BLPACR strains (20).

Generally, *H. influenzae* strains are highly susceptible to third-generation cephalosporins. The non-susceptibility rate of *H. influenzae* to third-generation cephalosporins was < 2% in the present study. This rate was much smaller than the rate reported in Iran (33.1%) (21) and Japan (49.4%) (22); however, it was similar to the rate of genital strains (5.5%) in China in 2015. In this regard, different infection sites may explain such an inconsistency. Typically, *H. influenzae* is sensitive to carbapenem; however, carbapenem-non-susceptible *H. influenzae* has also been reported in the literature (23). The present findings reported one *H. influenzae* strain non-susceptible to meropenem, whose mechanism is worthy of research in future studies.

This study showed the high prevalence of sulfamethoxazole-trimethoprim resistance (47.7%) among *H. influenzae* isolates; however, no significant difference was noticed between the present findings (47.7%, 306/641) in 2016 - 2019 and the previous ones (51.8%, 57/110) in 2015 (11). This might have been caused by the fewer applications of sulfamethoxazole-trimethoprim. Moreover, 10.2% of the *H. influenzae* isolates were resistant to azithromycin in this study. A significantly increased resistance was noticed during 2018 - 2019, which might be caused by the extensive use of azithromycin in respiratory infections in China. Furthermore, in the study, *H. influenzae* strains were all sensitive to levofloxacin, and 1.1% of *H. influenzae* strains were resistant to chloramphenicol. This is probably because these antibiotics are rarely used in children in China. MDR was observed in 41 cases (6.4%) of the 642 *H. influenzae* isolates. In line with the findings of the previous studies, the most prevalent resistance phenotype was ampicillin-sulfamethoxazole-trimethoprim-azithromycin resistance (15).

5.1. Conclusions

To the best of our knowledge, the present study represents the largest population-based study on *H. influenzae* vulvovaginitis among prepubertal girls in China. *H. influenzae* is considered as a common bacterial cause of vulvovaginitis in children in Zhejiang, China; hence, laboratories are recommended to routinely cover *Haemophilus* culture media for vulval specimens and consider the ampicillin resistance of *H. influenzae* in clinical management. A prominent strategy to prevent vulvovaginitis in prepubertal girls is to provide suggestions on hand hygiene and behaviors.

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Footnotes

Authors' Contribution: Study design: Liying Sun, Xuejun Chen, and Chunzhen Hua. Data analysis and interpretation and manuscript drafting: Mingming Zhou and Xuejun Chen. Strain identification and antimicrobial susceptibility test: Mingming Zhou, Chao Fang, and Jianping Li. Contributing equally to this study: Mingming Zhou and Liying Sun.

Conflict of Interests: The authors declare no conflict of interests.

Ethical Approval: The study was approved by the Research and Ethics Committee of The Children's Hospital, Zhejiang University School of Medicine (code: 2019-IRB-049).

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