Original Article

ISOLATION AND ANTIBIOGRAM PATTERN OF
HAEMOPHILUS INFLUENZAE ISOLATED FROM BRONCHIAL
WASHING OF PATIENTS UNDERGOING BRONCHOSCOPY

Ahmad Farajzadeh-Sheikh PhD*, Nasser Mosavy MSc, Heshmatollah Tavacol MD

Background – Haemophilus influenzae is the second most important causative agent of
pneumonia in outpatients. The aim of this survey was to perform isolation, to identify biotype,
serotype, and antimicrobial sensitivity pattern of Haemophilus influenzae in patients undergoing
bronchoscopy.

Methods – In this study 170 bronchial washing specimens were taken from patients 1 – 70
years old, and were cultured on blood agar, chocolate agar, cefsulodin chocolate agar, and Fildes
media. The presence of encapsulated bacteria was identified by Fildes medium neqrosin and
Congo red staining. Biotyping and serotyping were performed by serological methods.

Results – The results showed that 14 (8.23%) cases were positive for H. influenzae, of which
78.5% were unencapsulated. The cases were identified as I, II, III, and V biotypes. A statistically
significant relationship was found between unencapsulated H. influenzae and the age of the
subjects (p = 0.0345), and their occupation, e.g., cotton-beater and mine workers (p = 0.0196). From
isolated bacteria, 85.7% were sensitive to chloramphenicol and 71.42% to ampicillin and
cefotaxime.

Conclusion – Age and occupation are two risk factors for this bacterial respiratory, and the
prevailing biotypes in the region were I, II, III and V. Fortunately, some antibiotics are too high
degrees, effective on this agent.

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Keywords • antibiogram • bronchoscopy • Haemophilus influenzae

Introduction

Pneumonia is an important cause of mortality in children and the elderly.\textsuperscript{1, 2}
Haemophilus influenzae, Streptococcus pneumoniae, and Staphylococcus aureus are the
most important causes.\textsuperscript{3, 4} Haemophilus influenzae is an important cause of acute, recurrent and
persistent infection of the human respiratory tract.\textsuperscript{5} It is found in 8% of pneumonias.\textsuperscript{6} More
than 95% of the invasive infections are associated with type b encapsulated H. influenzae,\textsuperscript{7} which
accounts for a third of all bacterial pneumonias among 4-month to 4-year old children.\textsuperscript{8} The
incidence of this bacterium is reported to be 5 – 10 cases per 100,000 persons in USA of which 95%
are caused by type b.\textsuperscript{9, 10} The unencapsulated strains also play an important role in chronic
bronchitis, and are the second cause of pneumonia in adults.\textsuperscript{11} These strains are also an important
cause of lower respiratory tract infections in patients with cystic fibrosis.\textsuperscript{12}

We attempted to isolate and identify Haemophilus influenzae in patients suffering from
respiratory tract disorders and biotype, serotype, and do antimicrobial susceptibility testing on them.

Patients and Methods

Bronchial washings from 170 patients (41.7% females and 58.3% males) undergoing
bronchoscopy for various reasons at Imam Khomeini Hospital in Ahwaz, Iran, during 2000–
2001 were collected and studied for colonization of
<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Frequency No. (%)</th>
<th>Prevalence No. (%)</th>
<th>H. influenzae Encapsulated</th>
<th>Type b No.</th>
<th>Other type No.</th>
<th>Unencapsulated No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 – 10</td>
<td>24 (14.1)</td>
<td>2 (8.3)</td>
<td></td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>11 – 20</td>
<td>25 (14.7)</td>
<td>1 (4)</td>
<td></td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>21 – 30</td>
<td>21 (12.2)</td>
<td>0 (0)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>31 – 40</td>
<td>28 (16.4)</td>
<td>0 (0)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>41 – 50</td>
<td>15 (8.8)</td>
<td>2 (13.3)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>51 – 60</td>
<td>25 (14.7)</td>
<td>3 (12)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>32 (18.8)</td>
<td>6 (18.7)</td>
<td></td>
<td>0</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>170 (100)</td>
<td>14</td>
<td></td>
<td>1</td>
<td>2</td>
<td>11</td>
</tr>
</tbody>
</table>

Results

Fourteen cultures (8.23%) were positive for *Haemophilus influenzae*. (9.09% of males and 7.04% of females, P= NS). The highest frequency of *H. influenzae* (18.8%) was found in patients over 61 years of age. The frequency of *H. influenzae* correlated with increasing age ($p = 0.03458$). *H. influenzae* was isolated more frequently from cotton-beaters (25%) and mine workers (20%). In addition to smoking, a significant ($p = 0.0196$) relationship was found between occupation and prevalence rate of this bacterium in these cases (Table 2). Unencapsulated strains comprised 78.5% of isolated *H. influenzae*, of which 90.9% were found in patients over 40 years old. Three (21.4%) of the isolated *H. influenzae* were encapsulated strains, of which 2 were in a group under 20 years old, and a significant relationship was obtained between status of capsule and age ($p = 0.0286$). Of the encapsulated strains, only one was type b, isolated from a subject under the age of 10 years (Table 1). Biotypes I, III, and V comprised 92.8% (13) of isolates. Of these 10 were observed in patients older than 40 years, and 3 (2 biotype I and 1 biotype V) were seen in patients younger than 20 years old. We showed that there was not a statistically significant relationship between age, sex, and biotypes of this bacterium. Antimicrobial susceptibility testing showed that 85.7% of the cases were sensitive to chloramphenicol, 71.4% sensitive to ampicillin, and less than 50.0% to streptomycin, cephalexin, and cefazolin. The lowest sensitivity was equally observed with trimethoprim/sulfamethoxazole (28.5%) and cephradine (28.5%) (Table 3). The unencapsulated strains were more sensitive to ampicillin, amoxicillin, chloramphenicol, streptomycin, cefazolin, cefotaxime, cephalixin, and tetracycline than encapsulated strain. In contrast, the sensitivity of encapsulated strains to cephradine, ceftizoxime, and trimethoprim/sulfamethoxazole was more than the unencapsulated strains. Serotype-b was sensitive to ampicillin, chloramphenicol, ceftizoxime, and cephradine, and less than 10% resistant to amoxicillin, streptomycin, cephalixin, cefazolin, cephradine, trimethoprim/sulfamethoxazole, and tetracycline.

Discussion

We found a prevalence rate of 8.23% for *Haemophilus influenzae* in 170 cases of bronchial
Isolation and antibiogram pattern of *H. influenzae*

Bohte and colleagues in 1995 reported that prevalence rate of this bacterium was 8% in bronchitis cases. Butt and colleagues showed a prevalence rate of 7.3 – 25.0% in sputum of patients with bronchitis. The results of this study showed that there was no significant relationship between colonization of this bacterium and gender. Anderson et al in 1995 reported that both men and women are equally exposed to this bacterium. The prevalence rate of colonization of this organism is usually affected by age and behavioral factors (e.g., smoking). This study showed that a higher prevalence is seen in patients older than 61 years of age (Table 1), and this relationship was statistically significant. Therefore, older age can be considered a risk factor for contracting respiratory tract infection by this organism. We showed that in addition to smoking, there is also a significant relationship between occupation and this bacterium (Table 2). Cotton-beaters with 25% and mine workers with 20% prevalence showed the highest rate among our subjects. Cotton-beaters are usually exposed to dust containing bacteria, fungi, and other allergic substances over long periods of time. *H. influenzae* has been reported as an important pathogen for cystic fibrosis. In our study most of the *H. influenzae* isolates were unencapsulated, the majority of which were found in patients over 40 years old. Encapsulated strains were mostly seen in patients under the age of 20 (Table 1). We found a statistically significant relationship between respiratory tract disorder and unencapsulated *H. influenzae* strains, and that this had a significant relationship with age. Anderson and colleagues reported that unencapsulated *H. influenzae* was responsible for 95% of respiratory tract infection of which 62% were seen in adults. Smith-Vaughan

### Table 2. Prevalence of *H. influenzae* according to occupation and smoking.

<table>
<thead>
<tr>
<th>Occupation</th>
<th>Patients No. (%)</th>
<th>Frequency of smokers No. (%)</th>
<th>Total patients No. (%)</th>
<th>Smoker No. (%)</th>
<th>Non-smoker No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Student</td>
<td>29 (17.05)</td>
<td>0 (0)</td>
<td>1 (3.44)</td>
<td>0 (0)</td>
<td>1 (3.44)</td>
</tr>
<tr>
<td>Private business</td>
<td>45 (26.47)</td>
<td>8 (17.77)</td>
<td>1 (2.22)</td>
<td>0 (0)</td>
<td>1 (2.22)</td>
</tr>
<tr>
<td>Government staff</td>
<td>19 (11.17)</td>
<td>3 (15.78)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Mine worker</td>
<td>10 (5.88)</td>
<td>4 (40)</td>
<td>2 (20)</td>
<td>1 (25)</td>
<td>1 (16.66)</td>
</tr>
<tr>
<td>Car driver</td>
<td>8 (4.70)</td>
<td>5 (6.25)</td>
<td>1 (12.5)</td>
<td>1 (20)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Train driver</td>
<td>2 (1.17)</td>
<td>2 (0.00)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Cotton-beater</td>
<td>4 (2.35)</td>
<td>2 (50)</td>
<td>1 (25)</td>
<td>0 (0)</td>
<td>1 (25)</td>
</tr>
<tr>
<td>Housewife</td>
<td>17 (10)</td>
<td>3 (17.64)</td>
<td>3 (17.64)</td>
<td>0 (0)</td>
<td>3 (17.64)</td>
</tr>
<tr>
<td>Retired</td>
<td>30 (17.64)</td>
<td>6 (20)</td>
<td>4 (13.33)</td>
<td>0 (0)</td>
<td>4 (13.33)</td>
</tr>
<tr>
<td>Farm worker</td>
<td>6 (3.52)</td>
<td>2 (33.33)</td>
<td>1 (16.66)</td>
<td>0 (0)</td>
<td>1 (16.66)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>170 (100)</strong></td>
<td><strong>35 (20.58)</strong></td>
<td><strong>14 (8.23)</strong></td>
<td>2 (5.71)</td>
<td><strong>12 (8.88)</strong></td>
</tr>
</tbody>
</table>

### Table 3. Antimicrobial susceptibility pattern of isolated *H. influenzae* from patients.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Resistant</th>
<th>Intermediate</th>
<th>Sensitive</th>
<th>No. (%) of <em>H. influenzae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin 10 µg</td>
<td>3 (21.42)</td>
<td>1 (7.14)</td>
<td>10 (71.42)</td>
<td></td>
</tr>
<tr>
<td>Amoxicillin 25 µg</td>
<td>4 (28.57)</td>
<td>2 (14.28)</td>
<td>8 (57.14)</td>
<td></td>
</tr>
<tr>
<td>Cephalexin 30 µg</td>
<td>5 (35.7)</td>
<td>3 (21.43)</td>
<td>6 (42.85)</td>
<td></td>
</tr>
<tr>
<td>Cefazolin 30 µg</td>
<td>7 (50)</td>
<td>2 (14.28)</td>
<td>5 (35.71)</td>
<td></td>
</tr>
<tr>
<td>Cephradine 30 µg</td>
<td>6 (45.85)</td>
<td>4 (28.57)</td>
<td>4 (28.57)</td>
<td></td>
</tr>
<tr>
<td>Cefotaxime 30 µg</td>
<td>2 (14.28)</td>
<td>2 (14.27)</td>
<td>10 (71.42)</td>
<td></td>
</tr>
<tr>
<td>Cefotaxime 30 µg</td>
<td>3 (21.42)</td>
<td>2 (14.28)</td>
<td>9 (64.28)</td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol 30 µg</td>
<td>1 (7.14)</td>
<td>1 (7.14)</td>
<td>12 (85.7)</td>
<td></td>
</tr>
<tr>
<td>Tetracycline 30 µg</td>
<td>2 (14.58)</td>
<td>5 (35.71)</td>
<td>7 (50)</td>
<td></td>
</tr>
<tr>
<td>Trimethoprim + sulfamethoxazole (1.25 µg + 23.75 µg)</td>
<td>8 (57.14)</td>
<td>2 (14.28)</td>
<td>4 (28.57)</td>
<td></td>
</tr>
</tbody>
</table>

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et al\textsuperscript{21} reported that in the aging cases during the life time, immunity responsible for \textit{H. influenzae} increases and therefore encapsulated form changes to unencapsulated \textit{H. influenzae}. Of the three encapsulated cases, one belonged to serotype-b. Anderson et al reported that 88% of type b \textit{H. influenzae} were isolated from cases that were under 5 years old.\textsuperscript{20} In this study we showed that there was not a statistically significant relationship between gender and status of encapsulation. In two separate reports, Kroll et al\textsuperscript{22, 23} showed that status of capsule in \textit{H. influenzae} was affected by bacterium gene and immunity of patients, but did not correlate with sex. Our \textit{H. influenzae} isolates belonged to biotypes I, II, III, and V, the majority belonging to biotypes I, III, and V. We found that there was not a statistically significant relationship between age, sex, and biotype of this bacterium. Antimicrobial susceptibility pattern of the isolated \textit{H. influenzae} showed that 85.7% were sensitive to chloramphenicol and 71.4% to ampicillin and cefotaxime. Lower (28.5%) sensitivity rates were found for trimethoprim/sulfamethoxazole (Table 3). Until 1973, all strains of \textit{H. influenzae} were sensitive to ampicillin. In 1974 several resistant strains were reported, which produced a plasmid-borne beta-lactamase. In two studies the sensitivity of this bacterium to ampicillin was reported as 80.0% and 67.2%\textsuperscript{24, 25}. In 1988 Matthews et al\textsuperscript{26} showed that Chloramphenicol-resistant strains of this bacterium was less and related to production of chloramphenicol acetyltransferase. In this study, we compared the effect of antibiotics on encapsulated and unencapsulated \textit{H. influenzae} isolated from patients, and found that unencapsulated strains were more sensitive to antibiotics. In 1997 Butt et al\textsuperscript{19} reported that the unencapsulated \textit{H. influenzae} strains were sensitive to ampicillin (96.1%), amoxicillin (89.7%), and chloramphenicol (100%).

We found that age and occupation were two risk factors for this bacterium. Majority of colonization of this bacterium belonged to unencapsulated strain and I, III, and V biotypes. Chloramphenicol and amoxicillin greatly affected this agent, and unencapsulated strain had more sensitivity than encapsulated strain to the antibiotics.

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**References**

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