

RESEARCH NOTE

FIVE-YEAR STUDY OF ANTIMICROBIAL SUSCEPTIBILITY AND β -LACTAMASE PRODUCTION IN *HAEMOPHILUS INFLUENZAE*

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Abstract. We evaluated 582 *Haemophilus influenzae* isolates from patients between January 2000 and December 2004. Overall, 433 isolates were obtained from sputum and bronchial washings, 124 isolates were from pus, 19 isolates were from blood and 6 isolates from cerebrospinal fluid. *H. influenzae* was sensitive to amoxicillin/clavulanate, ampicillin/sulbactam, gentamicin, cefuroxime, ceftriaxone, cefotaxime, ciprofloxacin, ofloxacin, imipenem, meropenem (range 97-100%), chloramphenicol (75%), ampicillin/amoxicillin (52%), but resistant to trimethoprim-sulphamethoxazole. As for β -lactamase production, 48.4% of the isolates tested were positive.

INTRODUCTION

Haemophilus influenzae remains a major public health problem in the post *H. influenzae* type b (Hib) conjugate vaccine era, responsible each year for more than 3 million cases of invasive disease and 400,000 deaths worldwide (Peltola, 2000). This organism causes acute exacerbation of chronic bronchitis, acute sinusitis, otitis media, community-acquired pneumonia, septicemia and meningitis. Ampicillin or amoxicillin was the empirical treatment until recently (Jain *et al*, 2006). To date, β -lactamase production is regarded as the most common resistance mechanism to β -lactam antibiotics in this species (Jansen *et al*, 2006). In the early 1980s the first β -lactamase-negative, ampicillin resistant iso-

lates were reported. In Europe, the prevalence of these isolates has been demonstrated in many countries ranging from 2% to 20% (Fluit *et al*, 2005). The mechanism for resistance in β -lactamase-negative ampicillin resistant isolates is believed to be associated with a modification of a penicillin-binding protein and/or a decrease in cell wall permeability (Markowitz, 1980; Mandelman *et al*, 1984). The high cost of cephalosporins and the development of drug resistance due to the irrational use of antimicrobial agents are important in developing countries. It was reported that in Indian hospitals, at primary health centers trimethoprim-sulphamethoxazole was recommended for the treatment of children with respiratory tract infection and third-generation cephalosporins for the treatment of children presenting with meningitis (Jain *et al*, 2006).

We present here the *in vitro* activity of 14 antimicrobial agents against *H. influenzae* isolates. This data analysis is necessary for cost-effective treatment of *H. influenzae* infection

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and helpful for the solution of empiric therapy so that further development of drug resistance may be slowed.

MATERIALS AND METHODS

Bacterial isolates and identification procedure

A total of 582 *H. influenzae* isolates were collected from patients at Siriraj Hospital, Bangkok during the 5-year period of January 1, 2000-December 31, 2004. They were obtained from sputum, bronchial washings, blood, cerebrospinal fluid (CSF) and pus (from ears or sinuses). Only one isolate from each patient was collected to prevent the acquisition of duplicate isolates with the same antimicrobial resistance pattern.

Sputum was obtained when the patient was able to expectorate. Sputum was considered acceptable for culture if it contained > 25 polymorphonuclear cells and < 25 epithelial cells per low-powered field. Processing of sputum and other clinical specimens, including bacterial isolation and identification, was performed using growth factors X, V and XV according to standard microbiological techniques (Murray *et al*, 2003).

Antimicrobial susceptibility and β -lactamase testing

Susceptibility testing was performed using the Kirby-Bauer disk-diffusion method and the interpretive criteria used were the recommendations of the Clinical Laboratory Standards Institute (CLSI, 2006). The *Haemophilus* test medium (Oxoid, UK) was incubated overnight at 35°C for 18-24 hours in 5% CO₂. β -lactamase production by *H. influenzae* was detected by the nitrocefin method (O' Callaghan *et al*, 1972; Livermore and Brown, 2001). Nitrocefin was obtained as pure powder from Becton Dickinson (Oxoid, UK). A 500 μ g/ml solution was prepared by dissolving 10 mg of nitrocefin powder in 1 ml of dimethylsulphoxide (DMSO). Glass containers were used since DMSO degrades plastic. Colonies of the test

isolates were scraped from chocolate agar and suspended in 1 drop of nitrocefin solution on a glass slide. β -lactamase activity was indicated by the development of a pink to red color within 1-2 minutes. The color of a negative reaction was yellow.

RESULTS

In this study, there were 103, 116, 102, 117 and 144 isolates in 2000, 2001, 2002, 2003 and 2004, respectively (Table 1). Three hundred eleven patients (53.4%) were male and 271 (46.6%) were female. The M:F sex ratio was 1.15:1. The age range was 2 months-88 years old. The sources of these specimens are shown in Table 2. Overall, 433 isolates (74.4%) were obtained from sputum and bronchial washing, 124 isolates (21.3%) from pus, 19 isolates (3.3%) from blood, and 6 isolates (1.0%) from cerebrospinal fluid.

Table 1
Prevalence of *Haemophilus influenzae*.

Year	No. of isolates	%
2000	103	17.7
2001	116	19.9
2002	102	17.5
2003	117	20.1
2004	144	24.7
Total	582	100

Table 2
Sources of specimens.

Specimens	No. of isolates	%
Sputum	423	72.7
Pus from ears and sinuses	124	21.3
Blood	19	3.3
Bronchial washings	10	1.7
CSF	6	1.0
Total	582	100

Table 3
Antimicrobial susceptibility of *H. influenzae*.

Drugs	% S
Ampicillin/amoxicillin	52
Amoxicillin/clavulanate	98
Ampicillin/sulbactam	97
Cefotaxime	99
Ceftazidime	100
Ceftriaxone	100
Cefuroxime	97
Cefepime	100
Imipenem	99
Meropenem	99
Ciprofloxacin	100
Ofloxacin	100
Chloramphenicol	75
Trimethoprim-sulphamethoxazole	60

S = sensitive

From antimicrobial susceptibility testing (Table 3), we found that *H. influenzae* was sensitive to amoxicillin/clavulanate, ampicillin/sulbactam, all cephalosporins tested, imipenem, meropenem, ciprofloxacin, ofloxacin (range 97-100%), chloramphenicol (75%), ampicillin/amoxicillin (52%) and trimethoprim-sulphamethoxazole (60%). As for β -lactamase production, 48.5% of the isolates tested were positive. All β -lactamase positive isolates were resistant to ampicillin/amoxicillin. The prevalence of β -lactamase negative, ampicillin-resistant isolates was very low (0.01%).

DISCUSSION

This study reports a high prevalence of *H. influenzae*, 102-144 isolates per year, especially high in sputum (72.7%) followed by pus from the ears and sinuses (21.3%). This confirms previous reports that the respiratory tract is the most common site of infection caused by this organism (Daoud *et al*, 2006; Tristram *et al*, 2007). The sputum was a clinical specimen obtained from the lower

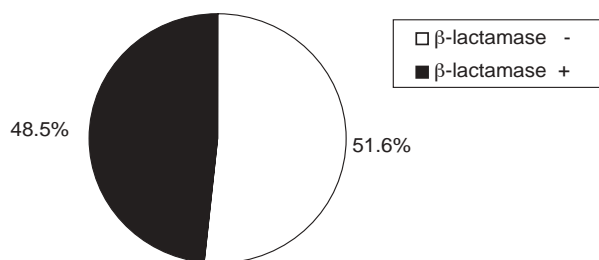


Fig 1—The percentage of β -lactamase production.

respiratory tract, but the pus in this study was obtained from the upper respiratory tract. Therefore, our *H. influenzae* isolates were from the lower respiratory tract, more than the upper respiratory tract.

Meningitis is a life threatening disease. During the 5-year study period, there were only 6 cases of meningitis caused by *H. influenzae*. The Hib vaccine is available in Thailand. However, due to the high cost of this vaccine and that it is not on the Expanded Program of Immunization (EPI) for Thailand, the Hib vaccine has limited use, mostly in private hospitals. We do not think this low incidence is a protective effect of the Hib vaccine. The low incidence of *H. influenzae* meningitis in this study may be similar to a study at a Beirut general hospital during the same time-period (2000-2004), which found only one isolate from the CSF in 2003 (Daoud *et al*, 2006). In contrast, a study in Salvador, Brazil from March 1996 to October 2000 had 524 cases of meningitis due to *H. influenzae* (Reis *et al*, 2002). This implies the distribution of *H. influenzae* worldwide varies geographically.

Using the disk diffusion method, we found 48% of *H. influenzae* were resistant to ampicillin, 40% resistant to trimethoprim-sulphamethoxazole and 25% resistant to chloramphenicol (Table 3). These resistance levels were higher than those of a previous report from many hospitals in Thailand (Bamrungrakul *et al*, 1994) which found 20.2% of *H. influenzae*

were resistant to ampicillin, 20% resistant to trimethoprim-sulphamethoxazole and 14.5% resistant to chloramphenicol. In India, of 316 *H. influenzae* type b isolates, 44% were ampicillin resistant (Jain *et al*, 2006). From a European surveillance study, a total of 578 *H. influenzae* isolates were prospectively collected and tested between October 2004 and April 2005 from patients with respiratory tract infections in various university hospitals in Austria, France, Germany, Ireland, Italy, the Netherlands, Poland, Portugal, Spain and the UK. Resistance to amoxicillin was found in 16.4% (Jansen *et al*, 2006). In Geneva, Switzerland, 18% of *H. influenzae* were resistant to amoxicillin (Jaecklin *et al*, 2006). In Brazil, 14% of *H. influenzae* were resistant to ampicillin (Castanheira *et al*, 2006). Our isolates had the highest level of resistance to ampicillin/amoxicillin, as well as trimethoprim-sulphamethoxazole and chloramphenicol. Worldwide susceptibility data from 8,523 isolates of *H. influenzae* (Tristram *et al*, 2007) showed that 17% were resistant to ampicillin, 16.8% resistant to amoxicillin, 17% resistant to trimethoprim-sulphamethoxazole and 1.9% resistant to chloramphenicol.

Our results concerning susceptibility data to β -lactamase stable penicillin (*eg*, amoxicillin/clavulanate), cephalosporins, fluoroquinolone and carbapenem were similar to many reports worldwide (Blosser-Middleton *et al*, 2003; Castanheira *et al*, 2006; Daoud *et al*, 2006; Jaecklin *et al*, 2006; Jain *et al*, 2006; Jansen *et al* 2006; Pichichero *et al*, 2006; Tristram *et al*, 2007). They were all excellent drugs against *H. influenzae* *in vitro*.

For our *H. influenzae* isolates, β -lactamase production was more commonly found than in reports from elsewhere, 48.5% compared to a worldwide range of 16.9-36% (Jones *et al*, 1997; Doern *et al*, 1999; Thornsberry *et al*, 1999; Credito *et al*, 2001; Jacobs *et al*, 2003). Isolates which were resistant to ampicillin mostly produced β -lactamase.

Surveillance of antimicrobial susceptibilities should be continued to evaluate trends and guide clinicians in the choice of the most appropriate empiric antimicrobial for the treatment of diseases caused by *H. influenzae*.

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