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Original Article

Antimicrobial Susceptibility of Invasive *Streptococcus pneumoniae* in Taiwan, 2008-2012

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Abstract

From January 2008 to December 2012, a total of 3,643 *Streptococcus pneumoniae* strains isolated from the confirmed cases of invasive pneumococcal disease were tested for the minimal inhibition concentrations (MIC) toward various antimicrobial agents. The susceptibility rates were 79.9% and 55.0% toward penicillins-type antimicrobial agents, amoxicillin and penicillin, respectively; 69.2% and 59.3% toward the third and the fourth generation cephalosporins, cefotaxime and cefepime, respectively; 94.7% and 95.3% toward quinolones-type antimicrobial agents, levofloxacin and moxifloxacin, respectively; 73.8%, 99.8%, 94.6% and 100.0% toward chloramphenicol, linezolid, telithromycin and vancomycin, respectively. The susceptibility rates were lower toward cabapenems-type antimicrobial agent, meropenem, and the sulfonamide-type antimicrobial agent, trimethoprim/sulfamethoxazole, 36.4% and 39.4%, respectively. The susceptibility rates toward erythromycin, tetracycline and clindamycin were the lowest, 9.6%, 9.0% and 24.2%, respectively. The susceptibility rates toward cefepime, clindamycin and meropenem continued to decline by year. The susceptibility rates toward amoxicillin and cefotaxime declined by year till 2011, and increased to 73.1% and 72.9% in 2012, respectively. In recent years, invasive infections caused by serotype 19A *S. pneumoniae* continued to increase and serotype 19A *S. pneumoniae* has become the most prevalent serotype in Taiwan. The non-susceptibility rates of serotype 19A *S. pneumoniae* towards amoxicillin, cefepime, cefotaxime, meropenem and penicillin were high, ranging from 81.6% to 97.2%. Among children younger than 5 years old, the highest proportion of infections was caused by serotype 19A *S. pneumoniae*, thus, the susceptibility rates toward the above mentioned antimicrobial agents among this age group were lower than those among other age groups, ranging from 13.0% to 54.8%. For strategies involved in prevention and treatment of invasive pneumococcal diseases, the result of this study

indicated that antimicrobial resistance of *S. pneumoniae* was another issue that is required a close attention, in addition to the usage of vaccines.

Key words: invasive pneumococcal disease, minimal inhibition concentration, serotype 19A

Introduction

Since October 2007, invasive pneumococcal disease (IPD) was listed as one of the fourth category notifiable diseases in Taiwan. The surveillance data of recent years revealed that the incidence rate is 3-4 cases per 100, 000 population for all age, and 13-20 cases per 100, 000 population among children younger than 5 years old [1]. In the United States, after the introduction of nationwide vaccination with seven-valent pneumococcal conjugate vaccine (PCV7), IPD incidence was reduced to 10-15 cases per 100, 000 population for all age and 20-30 cases per 100, 000 population among children younger than one year old [2]. The yearly incidence in other countries in Europe and America is about 10-100 cases per 100, 000 population [3].

Up to now, *Streptococcus pneumoniae* is still one of the most important pathogens that cause human respiratory infections. Since the first penicillin resistant *Streptococcus pneumoniae* strain was discovered in 1970s, the proportion of antimicrobial resistant strains continues to increase and multiple antimicrobial resistant strains continue to be discovered, resulting in a new threat to humankind [4, 5]. Although the surveillance data of recent years indicated that Taiwan is not one of those countries with high IPD incidence, however, in respect of antimicrobial resistance, the situation in Taiwan is always more serious than those developed countries in Europe and America [6, 7].

IPD is a vaccine preventable infectious disease. The current vaccines in the market are produced with the polysaccharides from the most prevalent serotypes among the 92 known serotypes of *S. pneumoniae*, and can efficiently prevent infections caused by the serotypes included in the vaccines. In fact, the introduction of these vaccines did result in the reduction of IPD case numbers in many countries. However, this was immediately accompanied by changes in the circulating serotypes, especially serotype 19A *S. pneumoniae*. Among *S. pneumoniae* that causes IPD, the proportion of serotype 19A *S. pneumoniae* continues to increase by year. Serotype 19A *S. pneumoniae* is also more resistant to many kinds of antimicrobial agents than other serotypes are [8-11]. Apparently, the new issue of vaccination has affected the antimicrobial resistance of *S. pneumoniae*.

Although the newly introduced 13-valent pneumococcal conjugate vaccine (PCV13) has included serotype 19A, whether this introduction will cause other new serotypes to increase in circulation, whether these new serotypes will have higher antimicrobial resistance, and whether there will be other new transmission mechanism of antimicrobial resistance, leading to even more serious problem in respect of antimicrobial resistance. All of these issues need to be considered for the prevention and treatment of IPD in future.

This study, based on long term surveillance of antimicrobial resistance of invasive *S. pneumoniae*, was carried out to monitor changes of antimicrobial resistance toward various antimicrobial agents, and to provide information for clinical treatment. The mechanism(s) of antimicrobial resistance and transmission will be further investigated in future.

Materials and Methods

Invasive *S. pneumoniae* isolates and confirmed cases of IPD

The case definition is based on the announcement for the fourth category of notifiable diseases. A case of IPD is defined as isolation of *S. pneumoniae* from a normally sterile body site, such as blood and cerebrospinal fluid (CSF). Any case that fits this definition will be required to report and to deliver the isolated *S. pneumoniae* strains to the Centers for Disease Control, Taiwan. From January 1, 2008 to December 31, 2012, a total of 3,643 invasive *S. pneumoniae* strains were collected from confirmed IPD cases nationwide. The demographic information, including year of infection, patient age and geographic region of residence, was provided by reporting hospitals for analysis.

Serotyping of *S. pneumoniae*

The capsular swelling method (Quellung reaction) was used for serotyping of *S. pneumoniae*. A loopful of purified bacterial suspension was mixed with a loopful of various antiserum (Statens Serum Institut, Copenhagen, Denmark) on microscopic glass slides, the obvious capsular swelling was observed under a light microscope when the serotype of the bacteria matched the specific type of antiserum used.

Antimicrobial susceptibility of *S. pneumoniae*

Minimal inhibition concentration (MIC) value was determined by the broth microdilution method using the BD Phoenix 100 Automated Microbiology System and Phoenix SMIC/ID9 panel (Becton Dickinson Diagnostic Systems, Sparks, MD, USA). Briefly, the bacterial suspension was adjusted to McFarland 0.5 by using the ID Broth. A drop of indicator and 25 μL of bacterial suspension was added to the AST Broth, and the mixture was poured into the detection panel within 20 minutes, followed by sealing of the panel and incubation in the System. *S. pnuemoniae* ATCC49619 was used as the control strain. Fifteen antimicrobial agents were tested, including amoxicillin, cefepime, cefotaxime, chloramphenicol, clindamycin, erythromycin, levofloxacin, linezolid, meropenem, moxifloxacin, penicillin G, telithromycin, tetracycline, trimethoprim/sulfamethoxazole and vancomycin. The result was interpreted according to the standards from the Clinical and Laboratory Standards Institute (CLSI), 2008 [12].

Results

Minimal inhibition concentration (MIC) and susceptibility toward various antimicrobial agents

In Taiwan, there were about 700 to 800 confirmed IPD cases every year. In this study, a total of 3,643 *S. pneumoniae* strains isolated from confirmed IPD cases between 2008 and 2012 were collected and tested for antimicrobial susceptibility. The result was interpreted according to the standards from the CLSI, and was listed in Table 1, including the range of MIC, MIC_{50}, MIC_{90}, and the percentages for susceptible, intermediate or resistant category. Most *S. pneumoniae* strains were highly resistant to tetracycline and macrolides-type antimicrobial agent, erythromycin, with susceptibility rates 9.0% and 9.6%, respectively. The susceptibility rates toward licosamides-type antimicrobial agent, clindamycin, cabapenems-type antimicrobial agent, meropenem, and sulfonamide-type antimicrobial agent, trimethoprim/sulfamethoxazole, were 24.4%, 36.4% and 39.4%, respectively. The susceptibility rate was 79.9% toward penicillin-type antimicrobial agent, amoxicillin, and was 55.0% toward penicillin, including 36.1% intermediate strains. The susceptibility rates toward the third and the fourth generation cephalosporins, cefotaxime and cefepime, were 69.2% and 59.3%, including 26.3% and 31.8% intermediate strains, respectively. The susceptibility rate toward chloramphenicol was 73.8%. Most *S. pneumoniae* strains had high susceptibility rates toward telithromycin, linezolid and quinolones-type antimicrobial agents, levofloxacin and moxifloxacin, 94.6%, 99.8%, 94.7% and 95.3%, respectively. No resistance toward vancomycin was observed.

Table 1. MIC and susceptibility rates of *S. pneumoniae* toward various antimicrobial agents

Antimicrobial agents	Percentage of all isolates (n=3,643)			MIC（μg/mL）		
	Susceptible	Intermediate	Resistant	MIC_{50}	MIC_{90}	Range
Amoxicillin	79.9%	8.8%	11.3%	1	>4	≦0.25 ~ >4
Cefepime	59.3%	31.8%	8.9%	1	2	≦0.5 ~ >2
Cefotaxime	69.2%	26.3%	4.5%	1	2	≦0.5 ~ >4
Chloramphenicol	73.8%	0.0%	26.2%	4	>8	≦2 ~ >8
Clindamycin	24.4%	0.3%	75.3%	>2	>2	≦0.03125 ~ >2
Erythromycin	9.6%	0.1%	90.3%	>4	>4	≦0.0625 ~ >4
Levofloxacin	94.7%	0.6%	4.7%	1	1	≦0.5 ~ >4
Linezolid	99.8%	0.0%	0.2%	≦1	≦1	≦1 ~ 4
Meropenem	36.4%	22.0%	41.6%	0.5	>0.5	≦0.0625 ~ >0.5
Moxifloxacin	95.3%	0.8%	3.9%	≦0.25	≦0.25	≦0.25 ~ >2
Penicillin G	55.0%	36.1%	8.9%	2	4	≦0.03125 ~ >8
Telithromycin	94.6%	3.9%	1.5%	≦0.0625	1	≦0.0625 ~ >2
Tetracycline	9.0%	2.3%	88.7%	>8	>8	≦0.5 ~ >8
Trimethoprim /Sulfamethoxazole	39.4%	11.5%	49.1%	2/38	>2/38	≦0.5/9.5 ~ >2/38
Vancomycin	100.0%	0.0%	0.0%	≦0.5	≦0.5	≦0.5 ~ 1

Susceptibility rates of *S. pneumoniae* toward various antimicrobial agents, by year

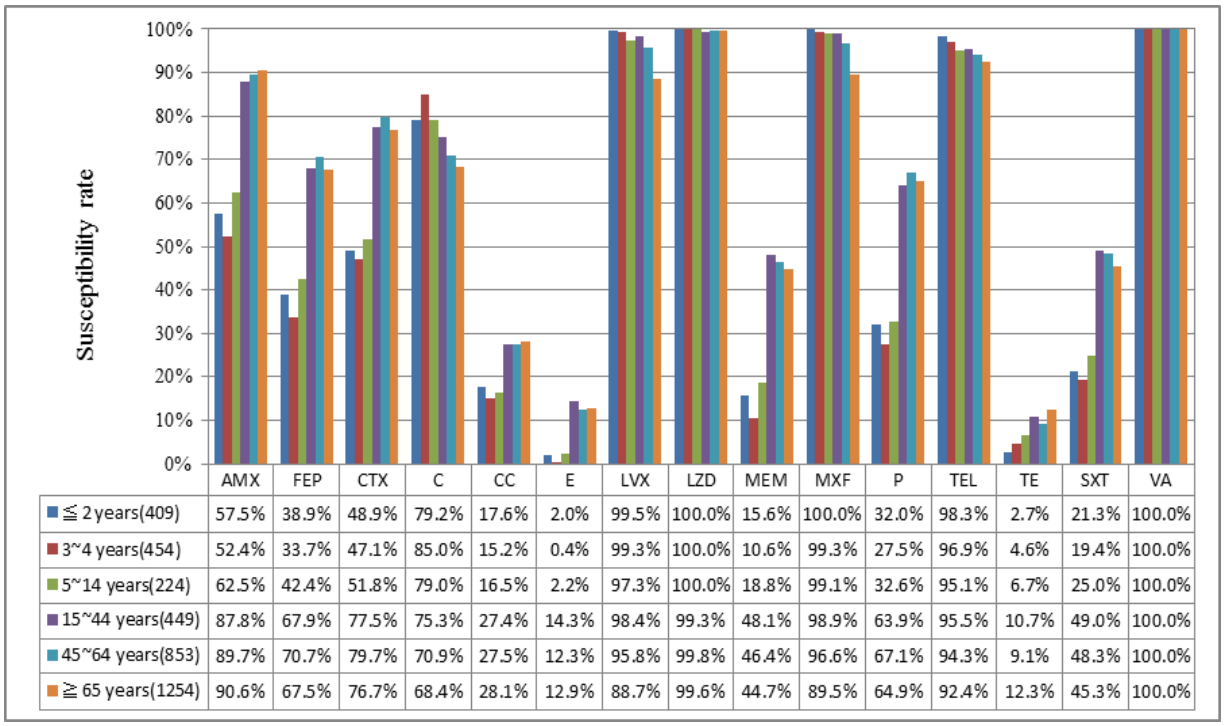
In order to explore changes in the susceptibility rates of *S. pneumoniae* toward various antimicrobial agents in recent years, we analyzed the susceptibility rates by year. Based on date of IPD onset, there were 768, 656, 714, 792, and 713 *S. pneumoniae* strains from 2008 to 2012, respectively. The susceptibility rates toward various antimicrobial agents were listed in Table 2. The susceptibility rates were around 95% toward levofloxacin, moxifloxacin and telithromycin, above 99% toward linezolid, and 100% toward vancomycin. The susceptibility rates toward chloramphenicol, erythromycin, tetracycline and trimethoprime/sulfamethoxazole did not show any increasing or decreasing trends, and fluctuated around 70.7%-77.3%, 8.4%-11.1%, 8.4%-10.2%, and 36.7%-40.5%, respectively. The susceptibility rates toward cefepime, clindamycin and meropenem continued to decline, from 70.2%, 30.1% and 42.6% in 2008 to 49.5%, 18.4% and 30.2% in 2012, respectively. A similar decreasing trend was observed toward amoxicillin and cefotaxime, from 89.7% and 71.9% in 2008 to 72.0% and 62.6% in 2011. However, the susceptibility rates increased back to 73.1% and 72.9% in 2012, respectively. Although the decreasing trend toward penicillin was less obvious, the susceptibility rate decreased from 57.0% in 2008 to 49.6% in 2011, and increased back to 58.9% in 2012.

Table 2. Susceptibility rates of *S. pneumoniae* toward various antimicrobial agents, by year

Antimicrobial agents	Year（number of isolate）					
	2008 (768)	2009 (656)	2010 (714)	2011 (792)	2012 (713)	All (3,643)
Amoxicillin	89.7%	86.6%	78.4%	72.0%	73.1%	79.9%
Cefepime	70.2%	61.9%	58.4%	56.4%	49.5%	59.3%
Cefotaxime	71.9%	71.3%	67.8%	62.6%	72.9%	69.2%
Chloramphenicol	73.7%	71.8%	70.7%	77.3%	74.9%	73.8%
Clindamycin	30.1%	25.3%	24.9%	23.1%	18.4%	24.4%
Erythromycin	11.1%	9.0%	8.4%	10.2%	8.6%	9.6%
Levofloxacin	94.7%	94.1%	94.5%	94.9%	94.8%	94.7%
Linezolid	99.7%	99.8%	99.6%	99.9%	99.6%	99.8%
Meropenem	42.6%	39.3%	35.0%	35.0%	30.2%	36.4%
Moxifloxacin	95.3%	95.3%	95.1%	95.3%	95.5%	95.3%
Penicillin G	57.0%	53.8%	55.7%	49.6%	58.9%	55.0%
Telithromycin	95.7%	93.9%	92.6%	97.0%	93.5%	94.6%
Tetracycline	9.0%	8.4%	8.5%	10.2%	8.6%	9.0%
Trimethoprim /Sulfamethoxazole	40.5%	36.7%	40.3%	38.4%	40.3%	39.4%
Vancomycin	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

Susceptibility rates of *S. pneumoniae* toward various antimicrobial agents, by age groups

Since young children and elderly persons are the high risk groups for IPD, we analyzed the susceptibility rates of *S. pneumoniae* toward various antimicrobial agents by six age groups, 0-2 years, 3-4 years, 5-14 years, 15-44 years, 45-64 years, and ≥65 years (Figure 1). Toward amoxicillin, cefepime, cefotaxime, clindamycin, erythromycin, meropenem, penicillin and trimethoprim/sulfamethoxazole, the susceptibility rates among children ≤14 years were apparently lower than those among individuals >14 years. Toward these antimicrobial agents, the lowest susceptibility rates were observed among children 3-4 years, 52.4%, 33.7%, 47.1%, 15.2%, 0.4%, 10.6%, 27.5% and 19.4%, respectively. These were significantly different from those among elderly ≥65 years, 90.6%, 67.5%, 76.7%, 28.1%, 12.9%, 44.7%, 64.9% and 45.3%, respectively (p<0.01). In contrast, the susceptibility rates toward chloramphenicol, levofloxacin, moxifloxacin and telithromycin among children ≤14 years were higher than those among individuals >14 years, but not statistically significant.



	AMX	FEP	CTX	C	CC	E	LVX	LZD	MEM	MXF	P	TEL	TE	SXT	VA
≦ 2 years(409)	57.5%	38.9%	48.9%	79.2%	17.6%	2.0%	99.5%	100.0%	15.6%	100.0%	32.0%	98.3%	2.7%	21.3%	100.0%
3~4 years(454)	52.4%	33.7%	47.1%	85.0%	15.2%	0.4%	99.3%	100.0%	10.6%	99.3%	27.5%	96.9%	4.6%	19.4%	100.0%
5~14 years(224)	62.5%	42.4%	51.8%	79.0%	16.5%	2.2%	97.3%	100.0%	18.8%	99.1%	32.6%	95.1%	6.7%	25.0%	100.0%
15~44 years(449)	87.8%	67.9%	77.5%	75.3%	27.4%	14.3%	98.4%	99.3%	48.1%	98.9%	63.9%	95.5%	10.7%	49.0%	100.0%
45~64 years(853)	89.7%	70.7%	79.7%	70.9%	27.5%	12.3%	95.8%	99.8%	46.4%	96.6%	67.1%	94.3%	9.1%	48.3%	100.0%
≧ 65 years(1254)	90.6%	67.5%	76.7%	68.4%	28.1%	12.9%	88.7%	99.6%	44.7%	89.5%	64.9%	92.4%	12.3%	45.3%	100.0%

Figure 1. Antimicrobial susceptibility rates toward various antimicrobial agents by age groups.

Antimicrobial agents included amoxicillin (AMX), cefepime (FEP), cefotaxime (CTX), chloramphenicol (C), clindamycin (CC), erythromycin (E), levofloxacin (LVX), linezolid (LZD), meropenem (MEM), moxifloxacin (MXF), penicillin G (P), telithromycin (TEL), tetracycline (TE), trimethoprim/sulfamethoxazole (SXT), vancomycin(VA).

Serotypes and antimicrobial susceptibility

IPD is a vaccine preventable infectious disease. The major serotypes of circulating *S. pneumoniae* in Taiwan were also included in the current vaccines. Vaccination is expected to prevent infections caused by these major circulating serotypes, however, would the antimicrobial susceptibility of these major circulating serotypes be affected? We analyzed the antimicrobial susceptibility of the eight most prevalent serotypes, 14, 3, 19A, 19F, 23F, 23A, 6B, 6A, and the other serotypes, representing 16.4%, 12.8%, 14.9%, 8.6%, 11.7%, 3.5%, 8.5%, 4.3% and 19.3% of all *S. pneumoniae* isolates, respectively. The non-susceptibility rates, including both intermediate and resistant susceptibility, of serotypes 19A and 19F *S. pneumoniae* were high toward amoxicillin, cefepime, cefotaxime, erythromycin, meropenem, penicillin, tetracycline and trimethoprim/sulfamethoxazole (Table 3), 91.7% / 37.1%, 89.5% / 63.3%, 81.6% / 50.8%, 98.7% / 98.7%, 97.2% / 93.6%, 89.3% / 76.0%, 97.1% / 96.8% and 95.0% / 95.2%, respectively, but low toward chloramphenicol, 1.5% and 12.1%, respectively. The non-susceptibility rates of serotype 19A were low toward levofloxacin and moxifloxacin, 0.7% and 0.6%, respectively. The non-susceptibility rates of serotype 23F were also high, especially toward meropenem, penicillin and trimethoprim/sulfamethoxazole, 97.0%, 71.2% and 86.7%, respectively. These were apparently higher than 7.0%, 14.7% and 9.3%, respectively, for serotype 23A from the same 23 group. In addition, serotype 23F had the highest non-susceptibility rates toward levofloxacin and moxifloxacin, 16.6% and 15.0%, respectively. The non-susceptibility rates of serotype 3 toward most antimicrobial agents were lower than those of other serotypes, except toward chloramphenicol and telithromycin, 73.3% and 19.1%, respectively, higher than other serotypes.

Table 3. Non-susceptibility rates toward various antimicrobial agents, by serotype

	Serotype (case number)								
antimicrobial agents	14 (597)	3 (465)	19A (544)	19F (313)	23F (427)	23A (129)	6B (310)	6A (156)	other (702)
Amoxicillin	13.1%*	0.0%	91.7%	37.1%	4.7%	1.6%	2.3%	0.0%	1.4%
Cefepime	48.2%	1.9%	89.5%	63.3%	56.2%	5.4%	23.5%	42.9%	15.8%
Cefotaxime	35.3%	0.2%	81.6%	50.8%	44.3%	2.3%	4.8%	13.5%	11.3%
Chloramphenicol	7.9%	73.3%	1.5%	12.1%	42.2%	7.0%	32.9%	54.5%	20.7%
Clindamycin	88.6%	76.8%	97.4%	52.7%	74.7%	80.6%	60.6%	80.8%	62.1%
Erythromycin	97.2%	80.2%	98.7%	98.7%	98.1%	96.9%	97.4%	93.6%	71.9%
Levofloxacin	6.5%	0.6%	0.7%	11.5%	16.6%	0.8%	2.6%	0.0%	4.8%
Linezolid	0.8%	0.0%	0.0%	0.0%	0.2%	0.0%	0.0%	0.0%	0.4%
Meropenem	89.8%	1.9%	97.2%	93.6%	97.0%	7.0%	65.2%	69.9%	30.6%
Moxifloxacin	5.9%	0.0%	0.6%	10.2%	15.0%	0.8%	1.0%	0.6%	4.6%
Penicillin G	52.9%	1.1%	89.3%	76.0%	71.2%	14.7%	28.4%	40.4%	17.2%
Telithromycin	5.5%	19.1%	4.6%	3.5%	2.6%	1.6%	1.3%	1.9%	2.4%
Tetracycline	82.2%	89.7%	97.1%	96.8%	93.4%	96.1%	96.1%	93.6%	86.9%
Trimethoprim/ Sulfamethoxazole	59.5%	2.4%	95.0%	95.2%	86.7%	9.3%	93.2%	41.7%	41.5%
Vancomycin	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

*Non-susceptibility rate represented both intermediate and resistant susceptibility; darkness of red color paralleled with the level of non-susceptibility rate toward individual antimicrobial agent.

Discussion

S. pneumoniae is one of the important pathogens for human respiratory infections. The invasive infection caused by this pathogen is a particular threat to health and life of humankind. Before vaccination was available for prevention of invasive infections caused by *S. pneumoniae*, usage of antimicrobial agents was the main treatment method. After widespread usage of antimicrobial agents, resistant *S. pneumoniae* gradually arose to circulate worldwide. Therefore, the current strategy for prevention and treatment of invasive pneumococcal infection should be based on monitoring of serotypes of *S. pneumoniae* for vaccine policy-decision making as well as on surveillance of antimicrobial resistance of *S. pneumoniae* for clinical treatment.

In 2008, the CLSI changed the interpretation criteria for antimicrobial susceptibility toward penicillin, cefepime and cefotaxime into meningitis and non-meningitis criteria [12]. For penicillin, the non-meningitis criteria are MIC ≤2μg/mL, 4μg/mL, and ≥8μg/mL for susceptible, intermediate, and resistant, respectively. For cefepime and cefotaxime, the non-meningitis criteria are MIC ≤1μg/mL, 2μg/mL, and 4μg/mL for susceptible, intermediate, and resistant, respectively. Therefore, the antimicrobial susceptibility rates reported in the literatures before and after the new criteria would be expected to be different [13]. The susceptibility rate toward penicillin was 32.2% in our earlier report of the surveillance of IPD in Taiwan in 2006 [14], much lower than 55.0% in the current report. This difference was contributed mainly by changes in the interpretation criteria. In the same earlier report, the susceptibility rates toward cephalosporins-type antimicrobial agents, cefotaxime and ceftriaxone, were 85.8% and 92.9%, respectively [14]. In our current report, the susceptibility rate toward cefotaxime was 69.2%, and toward fourth generation cephalosporins, cefepime, was only 59.3%, indicating that the susceptibility rates toward cephalosporins-type antimicrobial agents markedly declined. With a detailed analysis of the susceptibility rates in recent years, the susceptibility rate toward cefotaxime decreased from 71.9% in 2008 to 62.6% in 2011, and the susceptibility rate toward cefepime decreased from 70.2% in 2008 to 49.5% in 2012, revealing the same decreasing trends toward cephalosporins-type antimicrobial agents. The increase in the susceptibility rate toward cefotaxime back to 72.9% in 2012 deserves a continuous surveillance to see whether the susceptibility is really reversed, and whether these resistant *S. pneumoniae* strains are related. *S. pneumoniae* strains isolated from age groups 0-2 years, 3-4 years, and 5-14 years were significantly less susceptible to penicillin, cefotaxime or cefepime than isolates from other age groups ($p<0.01$). Furthermore, after wide introduction of pneumococcal conjugate vaccines in many countries, the population infected by serotype 19A *S. pneumoniae* markedly increased, especially among young children [15]. The susceptibility rates of serotype 19A *S. pneumoniae* toward cefotaxime and cefepime were less than 20%, far lower than those of other serotypes. It will require further investigation to reveal whether certain strains and/or certain mechanisms of antimicrobial resistance are circulating among these age groups.

Serotype 19A *S. pneumoniae* had high non-susceptibility rates toward most antimicrobial agents, however, the susceptibility rates toward levofloxacin and moxifloxacin were more than 99%, apparently higher than around 95% for all isolates. The susceptibility rate toward chloramphenicol was 98.5% for serotype 19A, in contrast to 26.7% for the generally less resistant serotype 3, suggesting that certain correlation exists between serotypes and antimicrobial resistance. The proportion of serotype 19A *S. pneumoniae* was 5.5%, 6.4%. 15.7%, 21.0% and 25.5% in 2008-2012, respectively. Among children <5 years old, the proportion of serotype 19A was 60.5%, leading to higher antimicrobial resistance and more serious drug resistance problem among this age group. Before serotype 19A became highly prevalent, the most prevalent serotypes 14, 23F, 19F and 6B all had higher resistance toward penicillin and most other antimicrobial agents [10, 14]. In our study, besides serotype 19A, serotypes 14, 23F and 19F still showed high resistance. However, serotype 6B showed higher resistance only toward trimethoprim/sulfamethoxazole, but not toward other antimicrobial agents. Since the isolation of serotypes 6C and 6D came from serotypes 6B and 6A, respectively, whether the antimicrobial resistance was affected needs to be further evaluated.

In Taiwan in recent years, the incidence of invasive pneumococcal disease did not show much change, however, the prevalent serotypes did change after the introduction of vaccination. The government responded it with the introduction of new vaccination policies, in the hope to reduce occurrence of IPD. In the meantime, the introduction of vaccines and the treatment with antimicrobial agents has resulted in drug resistance problem for *S. pneumoniae*. In our current study, the non-susceptibility rates of *S. pneumoniae* that caused IPD in this past five years varied toward certain antimicrobial agents (erythromycin, levofloxacin, linezolid, moxifloxacin, telithromycin, tetracycline, trimethoprim/sulfamethoxazole and vancomycin), continued to increase toward certain antimicrobial agents (cefepime, clindamycin and meropenem), or first increased followed by a decrease toward certain antimicrobial agents (amoxicillin, cefotaxime, chloramphenicol and penicillin). In addition, serotype 19A *S. pneumoniae*, with the main risk group among children <5 years old, continued to increase and resulted in more serious drug resistance problem than other serotypes. The effect of introduction of PCV13, including prevention of infections caused by serotype 19A, decrease in transmission of antimicrobial resistant strain, and further decrease in antimicrobial resistance, awaits further surveillance of antimicrobial susceptibility to address these issues.

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The Epidemiology, Clinical Manifestations and Prognosis of Invasive *Haemophilus Influenzae* Type b Infection in Taiwan, 2000-2012

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Abstracts

During 2000 to 2012, a total of 272 confirmed cases of invasive *Haemophilus influenzae* type b infection (hereinafter referred to as Hib) were identified, of which male were 176 cases (64.7%) and female were 96 cases (35.3%), the male to female ratio was 1.83:1. Among the confirmed cases, children aged less than 5 years old were 159 cases (58.5%) which accounted for the largest number. The high season was winter (41.5%). The most common clinical manifestations were pneumonia (44%), followed by bacteremia (21%) and meningitis (16%); the patients with meningitis had the highest rate to develop sequelae.

In recent years, the relevant vaccines have been introduced to Taiwan; the incidence of disease was significantly reduced. It has reduced to 0.01 cases per 100,000 population in 2012. Compared to developed countries in Europe and the United States, Taiwan is a low incidence country.

Keywords: Haemophilus, *Haemophilus influenzae* type b infection, meningitis

Introduction

Haemophilus influenzae is a gram-negative coccobacillus. It can be divided into encapsulated and non-encapsulated. Non-encapsulated organism is unable to subtype and encapsulated strains can be divided into six serologic types, a - f, of which b type is the most common cause of severe infant disease [1, 5, 7].

Hib is a serious infectious disease, mainly occurs in children aged less than 5 years. It causes invasive disease such as meningitis, bacteremia, pneumonia, cellulitis, bacterial arthritis, osteomyelitis, and epiglottis, etc. The incidence is considerably different in different regions. The most common presentation of invasive Hib disease is meningitis, which accounted for 50%-65%. The mortality rate is about 2% to 5%. About 15-30% survivors will develop sequelae such as hydrocephalus, encephalitis, ataxia, hearing damage, mental retardation and paralysis [1, 6, 8, 9]. It not only causes severely impact on personal health, but also increases the burden on family as well as the economic costs of the society and country. The conjugated Hib vaccine has been introduced to Taiwan since 1996, and five-in-one vaccine which containing the ingredients of conjugated Hib vaccine is incorporated into Taiwan's routine vaccination. The incidence of Hib decreases significantly. This study is to investigate the epidemiology, clinical manifestations and prognosis of Hib in recent years.

Materials and Methods

1. Study subjects:

In July 1999, the Taiwan Centers for Disease Control (Taiwan CDC) had listed Hib as the third category of notifiable infectious disease, and all people is the monitoring subjects. The medical institutes have to inform cases within one week in accordance with the provision. Whenever the *Haemophilus influenzae* is isolated from the blood, cerebrospinal fluid, and other normal sterile site from suspected cases, the notification should be done as soon as possible. The isolates have to send to Taiwan CDC for further serotype identification to determine the infection cases. The study subjects are the reported and confirmed cases that are retrieved from the Notifiable Reporting System at Taiwan CDC during January 2000 to the end of December 2012.

2. Case definition [2]:

A. Clinical requirements:

Invasive diseases caused by *Haemophilus influenzae* type b with clinical manifestations include meningitis, bacteremia, pneumonia, cellulitis, bacterial arthritis, osteomyelitis, and epiglottis, etc.

B. Laboratory requirements:

Normal and sterile specimens (blood, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid) are used to isolate and identify *Haemophilus influenzae* type b.

C. Notification definition:

In accordance with the clinical presentations, and the results of test are in line with the laboratory requirements.

D. Laboratory procedure:

Normal and sterile specimens (blood, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid) are used to isolate and identify *Haemophilus influenzae* type b. The procedures are as following:

(a) Conduct gram stain to confirm whether it is the negative coccobacilli.

(b) Conduct oxidase and catalase test, if both results are positive, then proceed the X, V , XV growth factor tests and API- NH (Biomérieux, France) biochemical identification.

(c) Conduct the serum agglutination test to make sure whether it is type b [3].

E. Confirmation of case definition: A confirmed case is a case with matched clinical and laboratory requirement.

3. Data source and analysis:

The data sources are from Epidemic Data Storage BO (business objects) System and Notifiable Infectious Disease Reporting System (Infectious Disease Epidemic Investigation System) at Taiwan CDC. Whenever a suspected case of Hib is found, medical personnel has to complete or use internet to fill in "Infectious Disease cases (including suspected cases) reporting sheet", and then send a report to local health authorities as well as Taiwan CDC. When the reported case is confirmed, the local public health personnel must visit the

case within 48 hours and complete the "Invasive *Haemophilus influenzae* type b infection Investigation Sheet". They must visit the case again within 3-6 months after onset to check if any sequela. The obtained information was analyzed and charting with Excel software. The results were mainly in descriptive statistics.

In this study, the denominator of the incidence per 100,000 population was taken from the mid-year population from Statistics Annual Report of the Ministry of Interior. The vaccination information was from "National Immunization Management Information Database System (hereinafter referred to as NIIS) of Taiwan CDC. The denominator of the database was the young children who were domiciled in Taiwan in the administrative data of the Ministry of Interior. This study collected data on birth cohort of 2000 to 2012 who received the primary Hib vaccine, the statistics of vaccination time was up to the December 31 of second year of each birth cohort.

The Epidemic Data Storage BO (business objects) System and Infectious Disease Epidemic Investigation System were built in 2006. Therefore, the number of reported cases and confirmed cases were collected from the year of 2000 to 2012, and the information of clinical manifestations, sequelae and deaths of the confirmed cases, were only available for analysis from January 2006 to the end of December 2012.

Results

1. Epidemiology:

A. Statistics of reported and confirmed cases:

A total of 1,058 Hib cases had been reported during 2000 to 2012. The number of cases per year ranged between 38 and 131, with an average of 81 cases. The confirmed cases were 272 cases, the number of cases per year ranged between 3 and 50, and with an average of 21 cases. The male cases were 176 cases (64.7%) and female were 96 cases (35.3%), the male to female ratio was1.83:1.

B. National incidence:

The incidence rate ranged from 0.22 to 0.01 cases/100,000 during the year of 2000 to 2012. The annual incidence rate had declined 95% as shown in Figure 1.

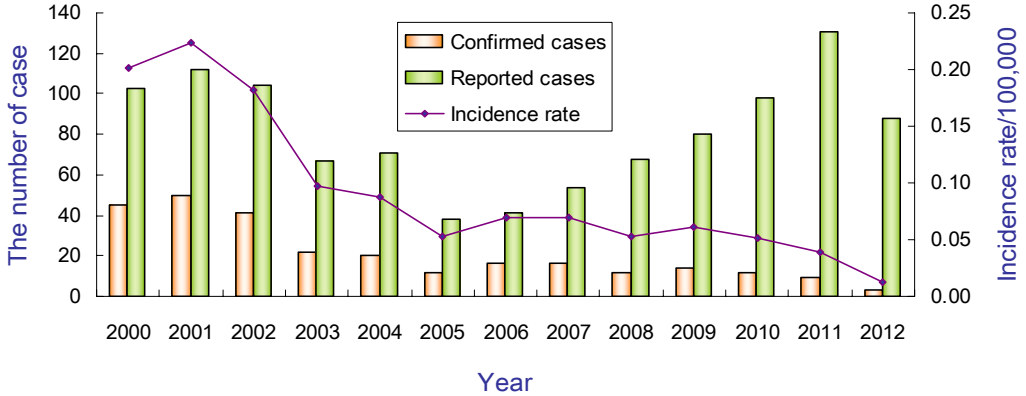

Figure 1. The incidence rate of invasive *Haemophilus influenzae* type b infection, Taiwan, 2000 to 2012

C. Age distribution

Among 272 confirmed cases, children aged less than 5 years were 159 cases (58.5%), which accounted for the largest number, followed by 5-19 years of 46 cases (16.9%), over the age of 65 years of 30 cases (11%), 40-64 years of 23 cases (8.5%) and 20-39 years of 14 cases (5%). The confirmed cases in all ages had decreased year by year (Figure 2).

D. Nationwide vaccination of *Haemophilus influenzae* type b

The conjugated *Haemophilus influenzae* type b vaccine had been introduced to Taiwan since 1996, and the self-pay five-in-one vaccine was introduced since 2002, but then the year of birth cohort vaccination was still less than 10%. In 2005, the self-pay five-in-one plus hepatitis B vaccine was introduced, the birth cohort vaccination rate was more than 50% and increased year by year since then. In March 2010, the five-in-one vaccine has been incorporated into national routine vaccination; the vaccination rate was more than 97% (Figure 3).

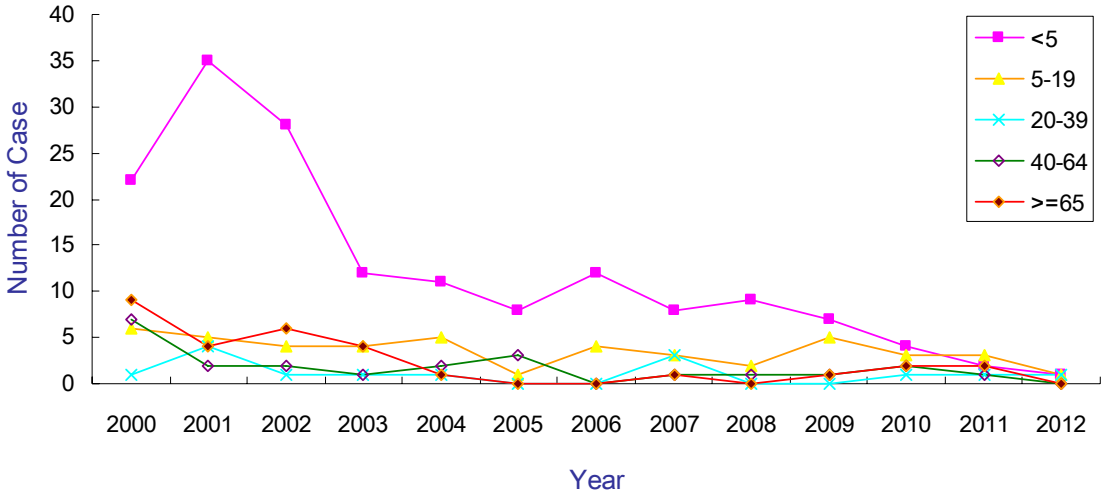

Figure 2. The age distribution of confirmed cases of invasive *Haemophilus influenzae* type b infection, Taiwan, 2000 to 2012

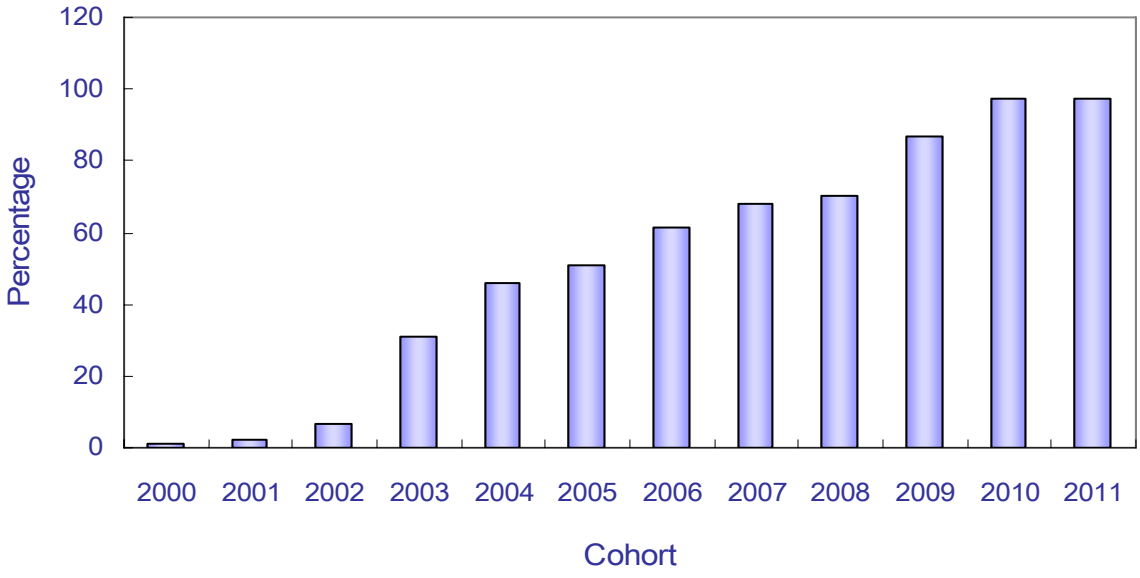

Figure 3. The primary dose vaccination rate of relevant vaccine that containing *Haemophilus influenzae* type b ingredient, 2000 to 2012 birth cohort

E. High season

The monthly confirmed cases of Hib were 62 cases (22.5%) from March to May in every spring, 55 cases (20.2%) from June to August in summer, 42 cases (15.4%) from September to November in autumn, and 113 cases (41.5%) form December to February in winter which had the highest numbers (Figure 4).

2. The clinical manifestations and prognosis of Hib

Based on the data of January 2006 to the end of December 2012, a total of 82 cases of Hib were indentified, including 14 cases that contained two sites of infection. The most common clinical manifestations of Hib were pneumonia, 42 cases (44%), followed by bacteremia, 20 cases (21%); meningitis, 15 cases (16%); the unknown cause was 9 cases (9%), epiglottitis was 6 cases (6%), and cellulitis, pericarditis, ankle arthritis, eye infection was 1 case (4%) each. Hib mainly occurred in children aged less than 5 years old. This study has found pneumonia is the most common clinical manifestation in all ages. The main causes of death were pneumonia and bacteremia, mostly were the elderly people over the age of 65 (60%). However, among15 cases of meningitis, children under 5 years old accounted for 11 cases, which was the main occurrence age group (Table 1).

As for the prognosis; among 82 Cases, 72 cases survived, 10 cases died, and the overall mortality was 12.2%. Among the 72 survived cases, 70 cases were fully recovered, 2 survivors had developed long-term sequelae.

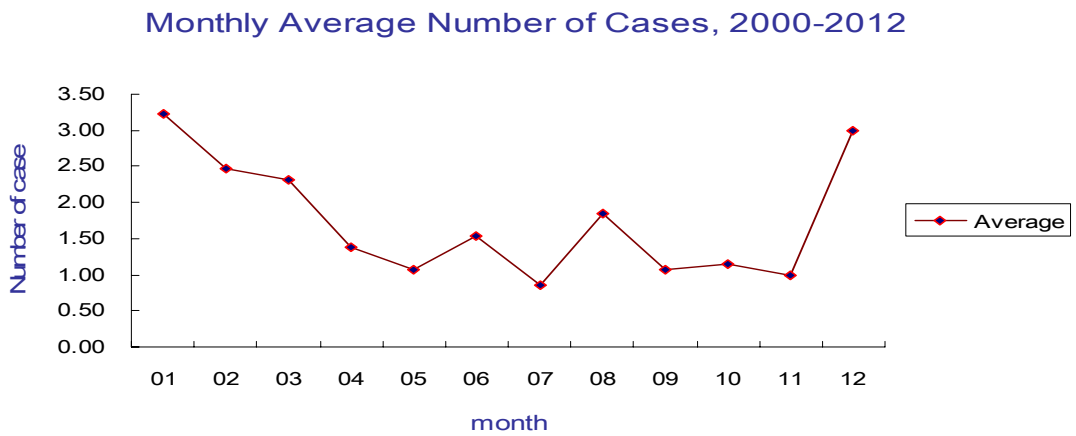

Figure 4. The monthly distribution of confirmed cases of invasive *Haemophilus influenzae* type b infections, 2000 to 2012

Table 1. The clinical manifestations and prognosis of invasive *Haemophilus influenzae* type b infection, 2006-2012

	Number of Case[a]			Death (%)		survivor (%)		Recovery (%)		Sequela (%)	
	Subtotal	<5yrs	≧5yrs	<5yrs	≧5yrs	<5yrs	≧5yrs	<5yrs	≧5yrs	<5yrs	≧5yrs
Clinical manifestations											
pneumonia	42	16	26	0(0)	5(19)	16(100)	21(81)	16(100)	21(100)	0(0)	0(0)
bacteremia	20	11	9	0(0)	3(33)	11(100)	6(67)	11(100)	6(100)	0(0)	0(0)
meningitis	15	11	4	0(0)	1(25)	11(100)	3(75)	9(82)	3(100)	2(18)	0(0)
epiglottitis	6	3	3	1(33)	0(0)	2(67)	3(100)	2(100)	3(100)	0(0)	0(0)
unknown fever[b]	9	7	2	0(0)	0(0)	7(100)	2(100)	7(100)	2(100)	0(0)	0(0)
others [c]	4	2	2	0(0)	0(0)	2(100)	2(100)	2(100)	2(100)	0(0)	0(0)

a. The cases number in the above table, 14 cases contained 2 sites of infection.

b. Unknown fever*: Unexplained fever and similar to the symptoms of upper respiratory tract.

c. Others*: Including one case of cellulitis, pericarditis, ankle arthritis and eye infection, respectively.

Discussion

Before the vaccine was introduced, Hib was the leading cause of bacterial meningitis and death among children under 5 years old. This disease becomes a vaccine preventable disease now and can be almost completely eliminated through routine vaccination [4]. The earliest Hib vaccine was developed in the 1970s. It contained purified capsular polysaccharide that coated over the surface of the bacteria. However, it was only for the children more than 18 months .Until 1990, the conjugated vaccine was developed to protect the babies more than 2 months old. The conjugated vaccine was an inactivated vaccine which combined capsular polysaccharide and protein [6], could reduce the carrier rate in the throat, thereby reducing the incidence of disease. Even the vaccination coverage rate was only 40-50%, it still could protect the unvaccinated people in the communities [4,7]. With the vaccination rate increased every year, the number of cases decreased significantly, and the incidence rate was reduced to 0.01 cases per 100,000 population in 2012. This study showed that the children under 5 years of age have a higher rate of Hib occurrence, but with the increased number of vaccination, herd immunity has also risen, and the incidence of b-type is relatively reduced. The effectiveness of vaccination is obvious. However, other types of *Haemophilus influenzae* cases do not diminish, there are still many reported cases in recent years. The majority of them are unable to sub-type or non-b type adult cases. Due to the vaccine was popularized and the incidence of children under 5 years of age dropped significantly, compared to the developed countries in Europe and the United States, Taiwan is a low incidence country.

As for the international epidemiology and vaccination policy, the polysaccharides and conjugated Hib vaccines had been introduced to the United States in 1985 and 1987 respectively [10], the incidence of children under 5 years decreased by 99%, and the incidence rate per 100,000 population was less than 1 case, the incidence rate/100,000 was 0.16 case during 2005 to 2009 [5]. As of 2009, the World Health Organization has introduced Hib vaccines into more than 89% countries in Europe region. Some Eastern European countries started to introduce the vaccine in support of GAVI (The Global Alliance for Vaccines and Immunization) in 2010. The incidence has been reduced to 0.39 case/100,000 in 2009 in European region, of which the highest incidence was Sweden (1.58 cases/100,000) and Norway (1.48 cases/ 100,000), followed by the UK (1.21 cases /100,000), Ireland (0.97 case/ 100,000) and Finland (0.88 case/ 100,000). The highest incidence was the children under 5 years of age (1.3 cases /100,000), followed by the elderly over 65 years (1.0 case /100,000) [11]. After the Hib vaccine had been introduced to Australia in 1993, the incidence dropped significantly, compared with the situation of unvaccinated, the incidence decreased by approximately 95% [6]. In Hong Kong and Singapore, Hib is an uncommon disease, the vaccine has not been incorporated into the routine vaccination [10,12].

Before the introduction of the vaccine, the incidence of Hib in Taiwan has been far lower than the rate after the introduction of the vaccine in Europe and USA. The clinical manifestations in Taiwan were also different from other countries. Our analysis discovered that

the common clinical manifestations of confirmed case were pneumonia, followed by bacteremia, meningitis, and epiglottis. It was slightly different from foreign studies that the most common clinical manifestations was meningitis [5,9,11]. It requires further study to identify the relations with the geographical environment, race, genetic in different countries. For the part of prognosis, the sequelae were mainly caused by the neuropathy of meningitis. Among 15 cases with clinical manifestations of meningitis, one case died, two cases produced sequelae, and the ratio was 14%. In addition, among 15 cases, 11 cases were infants and children under 5 years of age. It has shown that meningitis mainly occurred in children aged less than 5 years.

In addition to European region, Hib mainly occurs in children aged less than 5 years [11]. This study has found that the confirmed cases in more than 5 years old and adults still accounted for 40%, the data was similar to the research by Taiwan CDC in 2008 [13]. According to the literature [9, 14], except children, the high-risk groups of Hib were the patients who had low socioeconomic status, low immunity (splenectomy or dysfunction), and other chronic diseases (malignant tumor or chemotherapy, etc.). In this study, there were still 40% cases might be due to the effectiveness of the vaccine so the cases in children aged less than five years decreased, resulting in the increasing proportion of other age groups, or the cases themselves had disease history. It requires further studies to identify it.

The sequelae of Hib are mainly caused by the neuropathy of meningitis. It is also associated with the severity of the disease. Earlier study had shown that the 57% of meningitis survivors developed sequelae [13], but in this study is about 14%. The possible reasons might be the study in 2008 was to review the medical records and to investigate the reported and confirmed cases from August 1999 to the end of 2007, it was more complete than this study which was collecting the data from Notifiable Infectious Reporting System and Epidemic Investigation System, and the study period is relatively earlier and longer. Furthermore, the vaccination rate has increased in recent years, the incidence of cases dropped significantly, and medical quality improvement might also result in reduced the sequelae. In this study, both two cases with sequelae were caused by meningitis. Most of the studies which associated with the sequelae of Hib in foreign countries were focused on meningitis as the research subjects [15-20].

The international researches related to Hib, including a study conducted in southern Taiwan [15-20], adopted the hospital cases as the main targets for short-term or long-term follow-up, and reviewed the complete medical records of the cases as the basis for analysis. This study adopted the data from Epidemic Investigation System of Infectious Diseases, although the contents of the investigation could not be as detailed as clinical medical records, but the results of analysis were sufficient to present the epidemiology, clinical manifestations and prognosis of Hib that based on the population of Taiwan. In the future, access to the medical records for further analysis can increase the integrity of the information of clinical manifestations and prognosis.

Limitation

The Epidemic Data Storage BO (business objects) System and the Notifiable Infectious Disease Reporting System and Infectious Disease Epidemic Investigation System at Taiwan CDC was built in 2006. The information of confirmed cases' clinical manifestations, sequelae and deaths could only be retrieved from January 2006 to December 2012, so the number of cases was not many. Moreover, this study was limited by the underestimated incidence of the disease, mainly due to the physicians failed to suspect the patients, notify and perform the relevant tests while they make the diagnosis. Some hospitals had tested the Haemophilus strain and transferred to Taiwan CDC for further serotyping, but the poor transportation conditions affected on the test results. After the implementation of National Health Insurance, medical treatment becomes very convenient; the patients may be taking antibiotics at an early stage of the disease, which would also affect the correct diagnosis and test results. The above reasons may result in lower report of the incidence.

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