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Streptococcus pneumoniae bacteremia in southern Taiwan

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Streptococcus pneumoniae bacteremia was diagnosed in 33 patients between June 1999 and November 2000 at the Chang Gung Memorial Hospital-Kaohsiung in southern Taiwan. Antimicrobial susceptibility and serotyping of the clinical isolates were performed. Pneumonia was diagnosed in 19 patients, primary bacteremia in 13, and meningitis in one. The most common serotypes were types 14, 3, and 23F. Fourteen (42.4%) isolates of *S. pneumoniae* were non-susceptible to penicillin. High antimicrobial resistance rates were found to erythromycin (81.9%), tetracycline (69.7%), clindamycin (69.7%), trimethoprim-sulfamethoxazole (33.1%), and chloramphenicol (12.1%). The mortality was 42.4% and liver cirrhosis was an independent risk factor for mortality (odds ratio = 9.998; 95% confidence interval, 1.011-98.85; p=0.049). All isolated strains were covered by 23-valent the pneumococcal vaccine. Given the increasing prevalence of penicillin non-susceptible *S. pneumoniae* infection in the community, ongoing periodic monitoring of the evolutionary clinical situation is needed. Results of this study suggest that patients with liver cirrhosis should be inoculated with pneumococcal vaccine.

Key words: Antimicrobial susceptibility, bacteremia, serotyping, *Streptococcus pneumoniae*

Streptococcus pneumoniae infection is a global medical problem. Issues of particular concern are the increasing emergence of penicillin–non-susceptible and multidrug-resistant *S. pneumoniae* isolates [1,2], the high mortality associated with invasive pneumococcal bacteremia [3], and the method of designing a stratified pneumococcal vaccination program based on local demographic, clinical, and laboratory information. We reviewed all cases of *S. pneumoniae* bacteremia diagnosed at the Chang Gung Memorial Hospital-Kaohsiung (CGMH-KS) in the past 18 months. The study of clinical *S. pneumoniae* isolates was correlated with demographic, clinical, and microbiologic data.

Materials and Methods

Pneumococcal bacteremia was diagnosed in 33 patients at CGMH-KS, a 2300-bed medical center in southern Taiwan, during the period from June 1999 through November 2000. Clinical isolates of *S. pneumoniae* from each patient were subjected to microbiologic and antimicrobial susceptibility studies. The medical charts of all the patients were retrospectively reviewed for data including demographic characteristics, underlying diseases, clinical manifestations, antimicrobial therapy, laboratory findings, and clinical outcome. The appropriate use of the prescribed antibiotic in each case was determined based on the results of a susceptibility test. In cases of pneumococcal bacteremia, penicillin or a cephalosporin was considered appropriate if the isolate was susceptible or intermediately susceptible to penicillin. In cases of pneumococcal meningitis, penicillin was considered appropriate only if the isolate was susceptible, and a third-generation cephalosporin or vancomycin or combination of both was considered appropriate in all other cases.

Bacterial isolates

The various isolates of *S. pneumoniae* recovered from the 33 patients with bacteremia were identified based on the recognition of bacterial colony morphology on blood agar plate, gram-staining characteristics, susceptibility to optochin (Becton Dickinson, Cockeysville, MD, US), and bile solubility [4], and were confirmed by detection of *S. pneumoniae* antigen using Latex test (Murex Biotech, England).

Serotyping

All isolates were serotyped using the Quellung reaction. The 12-pooled diagnostic antisera, designated as A to I and P to T, (Statens Serum Institut, Copenhagen, Denmark) were obtained for serotyping. The serotypes

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of all isolates were determined using the simplified chessboard system as described by Sørensen [5].

Antimicrobial susceptibility testing

Antimicrobial susceptibility tests were performed on a clinical service basis using disc diffusion method in accordance with the National Committee for Clinical Laboratory Standards (NCCLS) [6]. For the disc diffusion susceptibility test, each isolated strain was inoculated into trypticase soy broth and incubated for 2 h at 35°C. Each isolate in broth was adjusted with 0.9% saline to obtain a turbidity visually comparable to that of the 0.5 McFacland nephelometer standard. A sterile cotton swab was dipped into the suspension and then streaked onto Mueller-Hinton agar supplemented with 5% sheep blood. The agar plate was allowed to dry for 3 to 5 min. The antimicrobial discs (BBL Sensi-Disc, Becton Dickinson) were attached and the inhibitory zones were observed after incubation at 35°C in 5% CO_2 for 20 to 24 h. All isolates were screened for susceptibility to penicillin G by the 1-μg oxacillin disc method. *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were used as control strains. Interpretations of the results were based on the NCCLS. The ranges of inhibitory zone determining susceptibility or resistance to the individual antibiotic were set according to the NCCLS. For the 1-μg oxacillin screening test, an inhibitory zone ≥20 mm indicates that the tested isolate was susceptible to penicillin G, whereas an inhibitory zone ≤19 mm indicates that the tested isolate was either intermediate or resistant to penicillin G. *S. pneumoniae* isolates with a ≤19-mm inhibitory zone in the 1-μg oxacillin screening test were further subjected to susceptibility testing using the E-test (AB Biodisk Solna, Sweden) according to the manufacturer's instructions. Isolates with minimum inhibitory concentration (MIC) ≤0.06 μg/mL were considered susceptible, ≥2 μg/mL were considered resistant, and those falling between 0.06 and 2 μg/mL, intermediate. Penicillin non-susceptible isolates refer to both intermediate and resistant strains unless otherwise stated. Multidrug resistance was defined as either intermediate or full resistance to at least 3 classes of antibiotics.

Statistical analysis

To determine the independent risk factors for mortality associated with pneumococcal bacteremia, potential risk factors were entered into a relational database for multivariate analysis (SPSS version 10.0). A p valve less than 0.05 was considered statistically significant.

Results

During the study period, 33 of 80 946 hospitalized patients were found to have *S. pneumoniae* bacteremia. No recurrent pneumococcal bacteremic episode was found. The hospital-based incidence of pneumococcal bacteremia was 40.8 per 100 000 admissions. The 33 patients included 18 males and 15 females with ages ranging from newborn to 84 years (mean, 43 ± 26 years). Seven patients aged less than 10 years, and 9 aged over 60 years.

Fever, chills, and cough with purulent sputum were found in 19 (57.6%) patients in whom pneumonia was eventually diagnosed. Dyspnea, altered consciousness, and shock were found in 14 (42.4%) patients. Among these 14 patients, meningitis was diagnosed in a 3-year-old child because cerebrospinal fluid additionally grew *S. pneumoniae*; primary *S. pneumoniae* bacteremia was diagnosed in the other 13 patients. Ceftriaxone was used to treat the pediatric patient with meningitis, who eventually survived. Among the 13 patients with primary bacteremia, either a first- or second-generation cephalosporin or vancomycin was prescribed. Fifteen (45.5%) patients had leukocytosis of more than 10 000 cells/μL, whereas the remaining 18 (54.5%) had normal white cell counts in peripheral blood.

The patients had a variety of underlying diseases. Seven (21.2%) patients had lung diseases including chronic obstructive pulmonary disease (4 cases), lung cancer (2 cases), and bronchiectasis (1 case); 6 (18.2%) patients had liver cirrhosis; 5 (15.2%) had malignancy including malignant lymphoma (3 cases) and colon cancer (2 cases); 3 (9.1%) had autoimmune diseases including systemic lupus erythematosus (2 cases) and dermatomyositis (1 case), and 5 (15.2%) had diabetes mellitus. None of the patients below 10 years of age had underlying disease.

The following 16 serotypes were identified among the 33 *S. pneumoniae* isolates: serotype 14 (8 patients), 3 and 23F (3 patients each), 4, 6A, 6B, 11, 18, and 9 (2 patients each), and 7, 10, 19F, 15, 9V, 22, and 23 (one patient each). *S. pneumoniae* isolates of serotypes 14 and 6A were commonly found in young patients, whereas serotypes 14, 4, and 9 were commonly found in elderly patients. The penicillin susceptibility tests revealed that 19 (57.6%) *S. pneumoniae* isolates were susceptible to penicillin G, and 14 (42.4%) were of intermediate susceptibility to penicillin G. No isolate was resistant to penicillin G. The antimicrobial resistance profile is shown in Table 1. Briefly, 3 (9%) isolates were susceptible to all test antibiotics, and 30 (92%) showed different resistance patterns: 2 (6%) were resistant to a single antibiotic, 3 (9%) to 2 classes of

Table 1. Serotypes and antimicrobial resistance patterns among 33 *S. pneumoniae* isolates

Antimicrobial agent	n (%)	No. of isolates with associated serotype															
		14	3	23F	4	6A	6B	11	18	9	10	19F	9V	15	7	23	22
P/E/Te/Cd/C/SXT	1 (3)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P/E/Te/Cd/SXT	5 (16)	3	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
P/E/Te/Cd	4 (12)	0	0	2	0	1	1	0	0	0	0	0	0	0	0	0	0
P/E/Cd/SXT	2 (6)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
P/E/Te/SXT	1 (3)	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
P/E/Te	1 (3)	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
E/Te/Cd/C/SXT	1 (3)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E/Te/Cd/C	3 (9)	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0
E/Te/Cd/SXT	3 (9)	2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
E/Te/Cd	4 (12)	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0
E/Te	1 (3)	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Te/Cd	1 (3)	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
E/Cd	1 (3)	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
E	1 (3)	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Te	1 (3)	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
None	3 (9)	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1
Total	33 (100)	8	3	3	2	2	2	2	2	2	1	1	1	1	1	1	1

Abbreviations: P = penicillin; E = erythromycin; Te = tetracycline; Cd = clindamycin; C = chloramphenicol; SXT = trimethoprim-sulfamethoxazole

antibiotic, 5 (15%) to 3 classes, 13 (39%) to 4 classes, 6 (19%) to 5 classes, and one (3%) to 6 classes. *S. pneumoniae* isolates of serotypes 14, 3, and 23F were predominant among all isolates.

Of all *S. pneumoniae* isolates intermediate to penicillin, serotypes 14 and 23F were the most common (5 and 3 patients, respectively). All patients received appropriate antibiotics, as cephalosporin was used when isolates were intermediately resistant to penicillin. Fourteen patients died of bacteremia. Their ages, underlying diseases, as well as serotypes and penicillin susceptibility of the *S. pneumoniae* isolates are shown in Table 2. Almost all of the bacteremic patients had underlying diseases except for 2 pediatric patients. The

Table 2. Clinical characteristics, penicillin susceptibility, and serotypes of isolates of 14 patients who died of *S. pneumoniae* bacteremia

Patient no.	Age (year)[a]	Sex	Underlying disease	*S. pneumoniae* isolate	
				Penicillin susceptibility[b]	Serotype
1	50	F	Diabetes mellitus	I	23
2	12	F	Dermatomyositis	I	14
3	7	F	Nil	I	14
4	68	M	Chronic obstructive pulmonary disease	I	23F
5	69	F	Malignant lymphoma Diabetes mellitus	I	14
6	56	M	Liver cirrhosis	I	14
7	45	M	Liver cirrhosis	I	9
8	1 d	F	Nil	S	11
9	46	M	Diabetes mellitus	S	7
10	66	M	Liver cirrhosis	S	22
11	55	M	Liver cirrhosis Diabetes mellitus	S	18
12	41	M	Liver cirrhosis Colon cancer	S	11
13	62	M	Chronic obstructive pulmonary disease	S	3
14	75	F	Liver cirrhosis	S	10

Abbreviations: I = intermediate; S = susceptible

[a]With the exception of patient 8 in which the newborn was 1-day-old.

[b]Based on results of the E-test.

overall mortality was 42.4%. Underlying diseases were found in 12 patients. Serotype 14 (4 patients) was the major type found in the mortal cases. One half of the strains isolated from these patients demonstrated intermediate penicillin resistance. The prognostic factors related to mortality in 33 patients with *S. pneumoniae* bacteremia are shown in Table 3. Liver cirrhosis was the only independent risk factor for mortality, with an odds ratio of 9.998, 95% confidence interval of 1.011 to 98.85, and p value of 0.049.

Discussion

Pneumococcal bacteremia is a serious invasive complication of *S. pneumoniae* infection. The population-based incidence of pneumococcal bacteremia varies with age groups and geographic areas [7,8]. Because all patients in this series were treated at the same medical center, selection of participants was inevitably biased by the referral pattern. As a result, the hospital-based incidence of pneumococcal bacteremia found in this study may not necessarily reflect the population-based incidence in southern Taiwan. Several predisposing factors to mortality were identified in this study. In comparison to other series [9-12], the mortality of pneumococcal bacteremia in this study was relatively high. One report has indicated that elderly patients with pneumococcal bacteremia have a high mortality [13]. While elderly patients and patients with underlying diseases such as chronic obstructive pulmonary disease, malignancy, diabetes mellitus, and abscence of prior vaccination have been reported to be prone to pneumococcal bacteremia in some studies [13,14], in this study liver cirrhosis was by far the most common underlying disease in patients who died of pneumococcal bacteremia. Hepatitis B is an endemic disease in Taiwan, and the frequency of liver cirrhosis is, therefore, inevitably high. Liver cirrhosis compromises the splenic function of the host, which may explain the high mortality in these patients. This study strongly suggests that patients with liver cirrhosis should receive pneumococcal vaccination. In this series, we found one case of pneumococcal meningitis in a newborn and another case of pneumococcal bacteremia in a 7-year-old patient, both of whom eventually died. Immature immunity is therefore also a predisposing factor to invasive pneumococcal bacteremia and contributed to a high mortality.

The overall mortality in this series was 42.4%, slightly higher than the 13% to 36% rate in previous reports [9-13]. Geriatric and pediatric patients of

Table 3. Prognostic factors related to mortality in 33 patients with *S. pneumoniae* bacteremia

Variable	Total no. of cases	No. of fatal cases (%)	p
Age (year)			0.456
≥16	7	3 (42.9)	
17-64	17	7 (41.2)	
≥65	9	4 (44.4)	
Sex			0.797
Male	18	8 (44.4)	
Female	15	6 (40.0)	
COPD			0.744
Yes	4	2 (50.0)	
No	29	12 (41.4)	
Diabetes mellitus			0.065
Yes	5	4 (80.0)	
No	28	10 (35.7)	
Liver cirrhosis			0.025
Yes	6	6 (100)	
No	27	8 (29.6)	
Malignancy			0.905
Yes	5	2 (40.4)	
No	28	12 (42.9)	
Penicillin susceptibility			0.450
Yes	14	7 (50.0)	
No	19	7 (36.8)	
Multidrug resistance			0.252
Yes	25	11 (44.0)	
No	8	3 (37.5)	

Abbreviation: COPD = chronic obstructive pulmonary disease

extreme ages with *S. pneumoniae* bacteremia have high mortality [15,16]. In this study, we also found a high mortality in middle-aged patients with underlying disease.

Previous studies have indicated that penicillin non-susceptible clinical isolates of *S. pneumoniae* have been increasing worldwide [1,2,17,18]. Fourteen (42.4%) isolates of *S. pneumoniae* in this study were penicillin non-susceptible. Chiou *et al* [18] reported that nasopharyngeal carriage of penicillin non-susceptible *S. pneumoniae* in children in Kaohsiung, the same geographic locale of this study, was as high as 71% from 1995 through 1997. The tendency of susceptibility of *S. pneumoniae* grown from blood culture in southern Taiwan is particularly worth following up. The 3 most common serotypes of *S. pneumoniae* in this series were 14, 3, and 23F. Although these serotypes were the same as those found in Taiwan in 1990 and 1993 [18,19], continuous periodic monitoring is important. The overall results of antimicrobial susceptibility tests in this study revealed erythromycin resistance to be 81.9%, tetracycline resistance 69.7%, clindamycin resistance 69.7%, trimethoprim/sulfamethoxazole resistance 33.3%, and chloramphenicol resistance 12.1%. Resistant rates to erythromycin and clindamycin were slightly higher compared with previous reports from Taiwan [2,3,19].

This study also found *S. pneumoniae* isolates of serotypes 14, 3, 23F, 4, 6A, 18, and 7 in patients with lung diseases, serotypes 14, 4, 9, 11, 18, and 22 in those with liver cirrhosis, serotypes 14, 23F, 4, 6, and 9 in malignant diseases, serotypes 14, 4, 18, 23, and 9V in metabolic diseases, and serotypes 14, 3, and 11 in autoimmune diseases. Further study with a much larger sample size is needed to determine whether a pneumococcal serotype exists, and if so, the nature of the specific underlying relationship. In this study, the difference in mortality between penicillin-susceptible and non-susceptible pneumococcal bacteremic patients was not significant, which is in accordance with previous reports [1,12]. The 33 strains isolated in this study were almost all covered by the currently used pneumococcal polysaccharide 23-valent vaccine. The overall coverage rate reached 100% if a serotype was included in the vaccine or if a serotype belongs to the same serogroup of the serotype found in the vaccine. Unfortunately, this vaccine has not been widely used in Taiwan.

In conclusion, given the astonishingly high percentage of children with nasopharyngeal carriage of penicillin non-susceptible *S. pneumoniae* in the community and the progressively increasing number of multidrug-resistant clinical isolates *S. pneumoniae*, continuous monitoring of the evolutionary status of *S. pneumoniae* is urgently needed. Restrictions on antibiotic prescription with tailored use by qualified infectious disease physicians is one effective approach to relieve the selective pressure on bacteria in general, and on *S. pneumoniae* in particular. Widespread pneumococcal vaccination for patients in high-risk groups is also important. Because liver cirrhosis is a risk factor for invasive pneumococcal infection with high mortality, liver cirrhosis patients should be vaccinated.

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