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ORIGINAL ARTICLE

Changing trends in antimicrobial susceptibility of *Streptococcus pneumoniae* isolates in Taiwan, 2006–2007

Chien-Feng Li [a,f], Meei-Fang Liu [a], Zhi-Yuan Shi [a,b,*], Po-Ren Hsueh [c], Chun-Hsing Liao [d], Tsrang-Neng Jang [e], Shih-Ming Tsao [f], Hsiang-Chi Kung [g], Gwo-Jong Hsu [h], Yu-Jen Cheng [i], Hsiu-Chen Lin [j], Yung-Ching Liu [k], Yin-Ching Chuang [l], Lih-Shinn Wang [m], Chih-Ming Chen [n]

[a] *Section of Infectious Diseases, Department of Internal Medicine, Taichung Veterans General Hospital, Taichung, Taiwan*
[b] *School of Medicine, National Yang-Ming University, Taipei, Taiwan*
[c] *Divisions of Clinical Microbiology and Infectious Diseases, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan*
[d] *Section of Infectious Diseases, Department of Internal Medicine, Far Eastern Memorial Hospital, Taipei, Taiwan*
[e] *Section of Infectious Diseases, Department of Internal Medicine, Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan*
[f] *Section of Infectious Diseases, Department of Internal Medicine, Chung Shan Medical University Hospital, Chung Shan Medical University, Taichung, Taiwan*
[g] *Section of Infectious Diseases, Department of Internal Medicine, National Taiwan University Hospital Yun-Lin Branch, Yunlin, Taiwan*
[h] *Section of Infectious Diseases, Department of Internal Medicine, Chiayi Christian Hospital, Chiayi, Taiwan*
[i] *Section of Infectious Diseases, Department of Internal Medicine, Changhua Christian Hospital, Changhua, Taiwan*
[j] *Department of Laboratory Medicine, Taipei Medical University Hospital, Taipei, Taiwan*
[k] *Section of Infectious Diseases, Department of Internal Medicine, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan*
[l] *Department of Internal Medicine, Chi-Mei Hospital, Tainan, Taiwan*
[m] *Section of Infectious Diseases, Department of Internal Medicine, Buddhist Tzu Chi General Hospital, Hualien, Taiwan*
[n] *Section of Infectious Diseases, Department of Internal Medicine, Tungs' Taichung MetroHarbor Hospital, Taichung, Taiwan*

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* Corresponding author. Section of Infectious Diseases, Department of Internal Medicine, Taichung Veterans General Hospital, 160 Section 3, Chung-Kang Road, Taichung 40705, Taiwan.
E-mail address: zyshi@vghtc.gov.tw (Z.-Y. Shi).

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Background: Multiple antibiotic-resistant clones of *Streptococcus pneumoniae* have spread throughout the world and continue to evolve under the selective pressure of antibiotics and vaccines. The aim of this study is to assess the susceptibility of *S. pneumoniae* isolates and to analyze the resistance trends in Taiwan.
Methods: Antimicrobial susceptibility tests were performed on 152 nonmeningeal isolates of *S. pneumoniae* that were collected from 13 different hospitals around Taiwan from 2006–2007. Tests were performed using the broth microdilution method according to recommendations of the Clinical and Laboratory Standards Institute.
Results: The minimal inhibitory concentrations (MIC_{50}/MIC_{90}) of penicillin, cefotaxime, vancomycin, and moxifloxacin were 0.5/1.0, 0.25/1.0, 0.25/0.5, and 0.06/0.12 μg/mL, respectively. The susceptibility rates of penicillin, cefotaxime, vancomycin, and moxifloxacin were 99.3%, 99.3%, 100%, and 98.7%, respectively. However, if the meningitis breakpoints were applied to these nonmeningeal isolates, the susceptibility rates of penicillin and cefotaxime were reduced to 18.4% and 76.3%, respectively. Compared with the findings from previous studies in Taiwan, our results show that the percentage of *S. pneumoniae* isolates with a penicillin MIC of 0.12–1.0 μg/mL increased from 43.3% in 1996–1997 to 73.7% in 2006–2007 ($p < 0.001$). The percentage of *S. pneumoniae* isolates with a cefotaxime MIC of 1.0 μg/mL increased from 11.3% in 1996–1997 to 23.0% in 2006–2007 ($p < 0.001$). Regarding the serial MIC intervals of the four antimicrobial agents, there was no significant difference between bacteremic and nonbacteremic isolates.
Conclusion: Although nonmeningeal *S. pneumoniae* isolates remained susceptible to penicillin, the proportion of isolates with a penicillin MIC of 0.12–1.0 μg/mL or cefotaxime MIC of 1.0 μg/mL increased during the past decade in Taiwan. The ever-increasing resistance of *S. pneumoniae* has a great impact on the treatment of meningitis.

Introduction

Penicillin-resistant *Streptococcus pneumoniae* (PRSP) was first reported in the late 1960s.[1] Subsequently, *S. pneumoniae* isolates with a higher levels of penicillin and multidrug-resistance (MDR) were found in South Africa in the late 1970s.[2] Higher prevalence rates of PRSP were reported in Europe and the USA in the 1990s.[3] The overall resistance to penicillin (minimal inhibitory concentrations [MIC] $\geq$ 2.0 μg/mL) was 23% (range: 6–54%), with the highest prevalence found in Spain and France (> 50%).[3] A report from the SENTRY Antimicrobial Surveillance Program (1999–2003) showed that the incidence of penicillin resistance (MIC $\geq$ 2.0 μg/mL) in *S. pneumoniae* was 14.7% in Europe, 12.7% in Latin America, and 15.9% in North America.[4] In North America, the prevalence of MDR *S. pneumoniae* increased from 5.7% in 1999 to 6.3% in 2003.[4] Clones of MDR *S. pneumoniae* have now been discovered worldwide.[5–7] The application of the new nonmeningeal breakpoints of penicillin (by CLSI-M100-S18 in 2008) had a great impact on susceptibility rates. Data from the SENTRY Antimicrobial Surveillance Program in 2006 were assessed using these new breakpoints.[8] Using a penicillin susceptibility breakpoint of $\leq$0.06 μg/mL, only 68% of isolates are susceptible to penicillin; in contrast, using a breakpoint of $\leq$2.0 μg/mL, 93% of isolates are susceptible.[8]

The prevalence of penicillin-insusceptible *S. pneumoniae* has been reported in Taiwan, with rates ranging from 56.4% (43% isolates with MIC of 0.12–1.0 μg/mL and 13.1% with MIC $\geq$ 2.0 μg/mL) in the late 1990s and 76.2% (50.7% with MIC of 0.12–1.0 μg/mL and 25.5% with MIC $\geq$ 2.0 μg/mL) in the early 2000s.[9–11] International clones of MDR *S. pneumoniae* have been identified in Taiwan since 1998.[12]

The resistance patterns of *S. pneumoniae* continue to evolve under the selective pressure of multiple factors (such as the use of antimicrobial agents,[13] clonal spread among hosts,[7] and vaccination).[14,15] The aim of this study is to assess the susceptibility of *S. pneumoniae* isolates, analyze resistance trends in Taiwan, and discuss the impact of revised nonmeningeal breakpoints recommended by the CLSI M100-S18.

Methods

S. pneumoniae isolates

One hundred and fifty-two unique isolates of *S. pneumoniae* were collected from 13 different hospitals in Taiwan, including nine medical centers (Buddhist Tzu Chi General Hospital: 4 isolates; Shin Kong Wu Ho-Su Memorial Hospital: 17 isolates; National Taiwan University Hospital: 31 isolates; Far Eastern Memorial Hospital: 25 isolates; Taichung Veterans General Hospital: 7 isolates; Chung Shan Medical University Hospital: 14 isolates; Changhua Christian Hospital: 9 isolates; Chi-Mei Hospital: 5 isolates; and Kaohsiung Veterans General Hospital: 5 isolates) and four local hospitals (Taipei Medical University Hospital: 7 isolates; Tungs' Taichung MetroHarbor Hospital: 4 isolates; Chiayi Christian Hospital: 11 isolates; and National Taiwan University Hospital, Yun-Lin Branch: 13 isolates), from 2006–2007. The sources of isolates included blood (27.6%),

sputum (47.3%), pus (18.4%), nasal discharge (4.6%), pleural fluid (0.7%), ear swab (0.7%), and eye swab (0.7%).

Broth microdilution

Antimicrobial susceptibility was assessed by broth microdilution according to the standard method approved by the CLSI.[16] The bacterial isolates were incubated overnight on 5% sheep blood agar plates. Inoculants were prepared from the bacterial colonies that formed on the 5% sheep blood agar plates, and then the colonies were suspended in 0.9% saline with a turbidity equivalent of 0.5 McFarland units. The suspension was further diluted within 15 minutes to give a final inoculum density of 5×10^5 CFU/mL in each test tube, containing 1.0 mL of antimicrobial agent that had been diluted in Mueller-Hinton broth with 5% lysed sheep blood. The following concentrations of penicillin and cefotaxime were used: 4 μg/mL, 2 μg/mL, 1 μg/mL, 0.5 μg/mL, 0.25 μg/mL, 0.12 μg/mL, 0.06 μg/mL, 0.03 μg/mL, and 0.01 μg/mL. The test tubes were incubated at 35°C in ambient air for 20–24 hours prior to determining MIC. *S. pneumoniae* ATCC 49619 was used as the control strain. The CLSI interpretive criteria[17] for MICs are as follow: (1) for nonmeningeal strains: penicillin susceptible: ≤ 2.0 μg/mL, intermediate susceptibility: 4.0 μg/mL, resistant: ≥8.0 μg/mL; cefotaxime susceptible: ≤ 1.0 μg/mL, intermediate susceptibility: 2.0 μg/mL, resistant: ≥4.0 μg/mL; moxifloxacin susceptible: ≤ 1.0 μg/mL, intermediate susceptibility: 2.0 μg/mL, resistant: ≥4.0 μg/mL; vancomycin susceptible: ≤ 1.0 μg/mL; (2) for meningeal strains: penicillin susceptible: ≤ 0.06 μg/mL, resistant: ≥0.12 μg/mL; cefotaxime susceptible: ≤ 0.5 μg/mL, intermediate susceptibility: 1.0 μg/mL, resistant: ≥2.0 μg/mL.

Statistical analysis

Analyses were performed by using the Statistical Package for the Social Science (version 15.1; SPSS Inc, Chicago, IL, USA) & SAS 9.1.3. The Kendall's tau-c test was used for comparisons of trends in the susceptibilities of *S. pneumoniae* isolates in Taiwan from 1996 to 2007, and the Pearson's chi-squared test was used for comparisons of the susceptibilities of 42 bacteremic and 110 nonbacteremic *S. pneumoniae* isolates. A difference was considered statistically significant if the two-tailed p-value <0.05.

Results

Susceptibility

The susceptibilities of the 152 *S. pneumoniae* isolates are shown in Table 1. The MIC_{50} and MIC_{90} of penicillin, cefotaxime, vancomycin, and moxifloxacin were 0.5/1.0, 0.25/1.0, 0.25/0.5, and 0.06/0.12 μg/mL, respectively. The susceptibility rates of penicillin, cefotaxime, vancomycin, and moxifloxacin were 99.3%, 99.3%, 100%, and 98.7%, respectively.

Comparisons of the susceptibilities of the 152 *S pneumonia* isolates using nonmeningeal and meningeal breakpoint criteria are shown in Table 1. According to the meningeal breakpoint, the susceptibility rates of penicillin and cefotaxime were 18.4% and 76.3%, respectively, while the resistance rates were 81.6% and 0.7%, respectively.

Cross-resistance between penicillin and cefotaxime

The cross-susceptibility and cross-resistance of penicillin and cefotaxime were analyzed and the results are listed in Table 2. The data generally show good categorical agreement between the MICs of penicillin and cefotaxime. However, there are some disagreements. For example, there were 8 isolates with penicillin MICs between 0.12–1.0 μg/mL, but the cefotaxime MICs ≤ 0.06 μg/mL; 10 isolates with penicillin MIC ≥ 2.0 μg/mL were identified, but their cefotaxime MICs were in the range of 0.12–1.0 μg/mL.

Trends in susceptibility

The susceptibility rates of the isolates in this study were assessed and compared with the results of two previous studies that were conducted in Taiwan.[9,18] Table 3 lists the changes in the susceptibility trends of *S. pneumoniae* isolates in Taiwan from 1996–2007. The serial percentages of *S. pneumoniae* isolates with penicillin MIC of 0.12–1.0 μg/mL were 43.3%, 38.7%, and 73.7%, respectively, whereas the serial percentages of *S. pneumoniae* isolates with penicillin MIC ≥ 2.0 μg/mL were 13.1%, 44.5%, and 7.9%, respectively. The percentage of *S. pneumoniae* isolates with penicillin MIC of 0.12–1.0 μg/mL increased from 43.3% in 1996–1997 to 73.7% in 2006–2007 ($p < 0.001$). The serial percentages of *S. pneumoniae* isolates with cefotaxime MIC of 1.0 μg/mL were 11.3%, 34.6 %, and 23.0%, respectively, while the serial percentages of *S. pneumoniae* isolates with cefotaxime MIC ≥ 2.0 μg/mL were 2.2%, 6.9%, and 0.7%, respectively. The percentage of *S. pneumoniae* isolates with cefotaxime MIC of 1 μg/mL increased from 11.3% in 1996–1997 to 23.0% in 2006–2007 ($p < 0.001$).

Comparisons of the susceptibilities of bacteremic and nonbacteremic isolates

Comparisons of the susceptibilities of 42 bacteremic and 110 nonbacteremic *S pneumonia* isolates are shown in Table 4. The susceptibilities of these isolates did not differ significantly ($p = 0.798$, 1, and 1 for penicillin, cefotaxime, and moxifloxacin, respectively).

Discussion

Of the 152 isolates, only 1 isolate (0.7%) was resistant to penicillin with an MIC of 4.0 μg/mL and only 1 isolate (0.7%) was resistant to cefotaxime with an MIC of 4.0 μg/mL. *S. pneumoniae* isolates with high resistance levels to extended-spectrum cephalosporins have been reported in the USA, the UK, Spain, and New Zealand since 1995.[19–22] Extended-spectrum cephalosporin-resistant *S. pneumoniae* isolates from the USA appear to have emerged from different clones.[19]A multidrug-resistant 19F clone of *S. pneumoniae* with high-level cefotaxime resistance was identified in New Zealand in 1997–1998.[22] *S. pneumoniae* isolates with high-level resistance to cephalosporin (1.4% of

Table 1 Antimicrobial susceptibilities of 152 *S. pneumoniae* isolates collected in Taiwan (2006–2007). The susceptibilities were interpreted according to the recommendations of CLSI M100-S18

Antimicrobial agents	Ranges of MIC (μg/mL)	MIC (g/mL)[a]		Susceptibility by nonmeningeal breakpoint[b]			Susceptibility by meningeal breakpoint		
		MIC_{50}	MIC_{90}	%S	%I	%R	%S	%I	%R
Penicillin G	<0.01–4	0.5	1	99.3	0.7	0	18.4	—	81.6
Cefotaxime	<0.01–4	0.25	1	99.3	0	0.7	76.3	23.0	0.7
Vancomycin	<0.01–1	0.25	0.5	100	0	0	—	—	—
Moxifloxacin	<0.01–2	0.06	0.12	98.7	1.3	0	—	—	—

[a] $MIC_{50/90}$, MIC of an antimicrobial agent inhibiting 50% or 90% of isolates, respectively.
[b] %S, percent susceptible; %I, percent intermediate susceptible; %R, percent resistant.

Table 2 Cross-susceptibility and cross-resistance analyses of the MIC intervals of penicillin compared with the MIC intervals of cefotaxime for 152 *S. pneumoniae* isolates

Isolates categorized by penicillin MIC (*n*)	Number (%) of isolates classified by cefotaxime MIC		
	≤0.06 μg/mL	0.12–1.0 μg/mL	≥2.0 μg/mL
≤0.06 μg/mL (28)	25 (89.2)	3 (10.8)	0
0.12–1.0 μg/mL (112)	8 (7.1)	104 (92.9)	0
≥2.0 μg/mL (12)	1 (8.3)	10 (83.4)	1 (8.3)

364 isolates) were also reported in another Taiwanese study in 2008.[18] The genotypes of *S. pneumoniae* isolates with high-level β-lactam resistance (penicillin MIC ≥ 4.0 μg/mL and cefotaxime ≥ 2.0 μg/mL) were then analyzed by multilocus sequence typing,[18] and three clones (Spain^{23F}-1, Taiwan^{19F}-14, and Taiwan^{23F}-15) were identified.

Determining the susceptibility breakpoint depends on the desired microbiological, pharmacokinetic, and/or pharmacodynamic clinical outcomes.[8] An intravenously administered 2 MU dose of penicillin every 4 hours would have a 99% likelihood of achieving serum concentrations greater than the MIC of 25% of the therapy interval when treating an isolate with an MIC of 2.0 μg/mL.[23] A meta-analysis suggested that increased mortality is not associated with an penicillin MIC ≥ 2.0 μg/mL for pneumococcal pneumonia.[24] These data support a penicillin-susceptible breakpoint ≤2.0 μg/mL for nonmeningeal isolates.[17] In this study, if the susceptibilities of all of the 152 nonmeningeal isolates were interpreted with the former penicillin-susceptible breakpoint ≤0.06 μg/mL,[25] only 18.4% of isolates would be susceptible to penicillin. In contrast, using the revised penicillin-susceptible breakpoint ≤2.0 μg/mL, 99.3% of isolates would be susceptible to penicillin. Using this revised nonmeningeal breakpoint of penicillin, the proportion of isolates susceptible to penicillin would be comparable with the proportion of isolates susceptible to the nonmeningeal breakpoint of cefotaxime (99.3%). In the USA from 2005–2006, the three surveillance networks (SENTRY, PROTEKT, and ABCs surveillance) reported that the susceptibility increased by approximately 25%, using the revised penicillin-susceptible breakpoint ≤2.0 μg/mL for nonmeningeal infections.[8]

The susceptibility of these nonmeningeal *S. pneumoniae* isolates remained high enough for the treatment of nonmeningeal infections. *S. pneumoniae*, though isolated from nonmeningeal sources, is also a potential pathogen of

Table 3 Trends in the susceptibility of 152 *S. pneumoniae* isolates in Taiwan (1996–2007)

Antimicrobial agents	MIC (μg/mL)	% of non-susceptible isolates			
		1996–1997[a] (*n* = 550)	2003–2006[b] (*n* = 364)	2006–2007[c] (*n* = 152)	*p*
Penicillin	≤0.06	43.6	16.8	18.4	<0.001
	0.12–1.0	43.3	38.7	73.7	
	≥2.0	13.1	44.5	7.9	
Cefotaxime	≤0.5	86.5	58.5	76.3	<0.001
	1.0	11.3	34.6	23.0	
	≥2.0	2.2	6.9	0.7	
Moxifloxacin	≤1.0	—	99.5	98.7	0.443
	2.0	—	0.2	1.3	
	≥4.0		0.3	0	
Vancomycin	2.0	0	0	0	—
	≥4.0	0	0	0	—

[a,b,c] Data adopted from references 9, 18, and this study, respectively.

Table 4 Comparison of the susceptibilities of 42 bacteremic and 110 nonbacteremic *S. pneumoniae* isolates

Antimicrobial agents	MIC (μg/mL)	Bacteremic isolates *n* (%)	Non-bacteremic isolates *n* (%)	*p*
Penicillin	≤0.06	9 (21.4)	26 (23.6)	0.798
	0.12—1.0	31 (73.8)	76 (69.1)	
	≥2.0	2 (4.8)	8 (7.3)	
Cefotaxime	≤ 1.0	42 (100.0)	109 (99.1)	1.000
	2.0	0 (0.0)	0 (0.0)	
	≥4.0	0 (0.0)	1 (0.9)	
Moxifloxacin	≤1.0	42 (100.0)	108 (98.2)	1.000
	2.0	0 (0.0)	2 (1.8)	
	≥4.0	0 (0.0)	0 (0.0)	
Vancomycin	≤1.0	42 (100.0)	110 (100.0)	—
	2.0	0 (0.0)	0 (0.0)	
	≥4.0	0 (0.0)	0 (0.0)	

meningitis. If the revised CLSI breakpoints for meningitis are applied to these nonmeningeal isolates, there will be a very dramatic impact on the susceptibility of *S. pneumoniae* to penicillin and cefotaxime. Resistance rates will be much higher if meningeal breakpoints are applied to these non-meningeal isolates. The following results are likely: only 18.4% of isolates will be susceptible to penicillin and 76.3% will be susceptible, 23% will be intermediately susceptible, and 0.7% will be resistant to cefotaxime. Categorical disagreement between the MICs of penicillin and cefotaxime was found in 22 isolates. This could be explained by alterations in penicillin-binding proteins that could result in different binding affinities for different β-lactams, e.g., penicillin and cefotaxime.[26]

Although 99.3% of the isolates were susceptible to penicillin using the nonmeningeal breakpoint, the percentage of *S. pneumoniae* isolates with penicillin MIC of 0.12—1.0 μg/mL increased from 43.3% (during the period of 1996—1997) to 73.7% (during the period of 2006—2007) ($p < 0.001$). The percentage of *S. pneumoniae* isolates with penicillin MIC of 2.0 μg/mL decreased from 13.1% (during the period of 1996—1997) to 7.9% (during the period of 2006—2007) ($p < 0.001$). Interpreting susceptibility by looking at just susceptibility or resistance will often mask changes in the MIC distribution and changes in resistance trends. Monitoring detailed MIC data is important for epidemiology studies and determining appropriate treatment strategies.

Regarding the serial MIC intervals of the four antimicrobial agents, there were no significant differences between bacteremic and nonbacteremic isolates. Only some major clones of antibiotic-resistant *S. pneumoniae* were found to be spreading in Taiwan; for example, Taiwan-23F, Taiwan-19F, Spanish-23F, Spanish-6B, Spanish-9 V, ST320 (19A), and ST902 (6A).[12,27] The most prevalent serotypes of invasive isolates also were attributed to these serotypes.[10,27] Therefore, the diversity of resistance patterns is limited.

In conclusion, although nonmeningeal *S. pneumoniae* isolates remain susceptible to penicillin, the proportion of isolates with a penicillin MIC of 0.12—1.0 μg/mL or cefotaxime MIC of 1.0 μg/mL has increased during the past decade in Taiwan. The ever-increasing resistance of *S. pneumoniae* has a great impact on the treatment of meningitis.

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