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High Prevalence of Antimicrobial Resistance among Clinical *Streptococcus pneumoniae* Isolates in Asia (an ANSORP Study)

Jae-Hoon Song,[1,2]* Sook-In Jung,[3] Kwan Soo Ko,[2] Na Young Kim,[2] Jun Seong Son,[1] Hyun-Ha Chang,[4] Hyun Kyun Ki,[1] Won Sup Oh,[1] Ji Yoeun Suh,[2] Kyong Ran Peck,[1] Nam Yong Lee,[5] Yonghong Yang,[6] Quan Lu,[7] Anan Chongthaleong,[8] Cheng-Hsun Chiu,[9] M. K. Lalitha,[10] Jennifer Perera,[11] Ti Teow Yee,[12] Gamini Kumarasinghe,[12] Farida Jamal,[13] Adeeba Kamarulzaman,[14] Navaratnam Parasakthi,[14] Pham Hung Van,[15] Celia Carlos,[16] Thomas So,[17] Tak Keung Ng,[17] and Atef Shibl[18]

Division of Infectious Diseases[1] and Department of Laboratory Medicine,[5] Samsung Medical Center, Sungkyunkwan University School of Medicine, Asian-Pacific Research Foundation for Infectious Diseases,[2] and Kyungbook National University,[4] Seoul, and Chonnam National University Hospital, Gwangju,[3] Korea; Beijing Children's Hospital, Beijing,[6] Shanghai Children's Hospital, Shanghai,[7] and Princess Margaret Hospital, Hong Kong,[17] China; Chulalongkorn University, Bangkok, Thailand[8]; Chang Gung Children's Hospital, Taipei, Taiwan[9]; Christian Medical College, Vellore, India[10]; University of Colombo, Colombo, Sri Lanka[11]; National University of Singapore, Singapore[12]; Universiti Putra Malaysia[13] and University Malaya,[14] Kuala Lumpur, Malaysia; University of Medicine and Pharmacy, Ho Chi Minh City, Vietnam[15]; Research Institute for Tropical Medicine, Manila, Philippines[16]; and King Saud University, Riyadh, Saudi Arabia[18]

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A total of 685 clinical *Streptococcus pneumoniae* isolates from patients with pneumococcal diseases were collected from 14 centers in 11 Asian countries from January 2000 to June 2001. The in vitro susceptibilities of the isolates to 14 antimicrobial agents were determined by the broth microdilution test. Among the isolates tested, 483 (52.4%) were not susceptible to penicillin, 23% were intermediate, and 29.4% were penicillin resistant (MICs ≥ 2 mg/liter). Isolates from Vietnam showed the highest prevalence of penicillin resistance (71.4%), followed by those from Korea (54.8%), Hong Kong (43.2%), and Taiwan (38.6%). The penicillin MICs at which 90% of isolates are inhibited (MIC_{90}s) were 4 mg/liter among isolates from Vietnam, Hong Kong, Korea, and Taiwan. The prevalence of erythromycin resistance was also very high in Vietnam (92.1%), Taiwan (86%), Korea (80.6%), Hong Kong (76.8%), and China (73.9%). The MIC_{90}s of erythromycin were >32 mg/liter among isolates from Korea, Vietnam, China, Taiwan, Singapore, Malaysia, and Hong Kong. Isolates from Hong Kong showed the highest rate of ciprofloxacin resistance (11.8%), followed by isolates from Sri Lanka (9.5%), the Philippines (9.1%), and Korea (6.5%). Multilocus sequence typing showed that the spread of the Taiwan^{19F} clone and the Spain^{23F} clone could be one of the major reasons for the rapid increases in antimicrobial resistance among *S. pneumoniae* isolates in Asia. Data from the multinational surveillance study clearly documented distinctive increases in the prevalence rates and the levels of antimicrobial resistance among *S. pneumoniae* isolates in many Asian countries, which are among the highest in the world published to date.

The global emergence of in vitro antimicrobial resistance in *Streptococcus pneumoniae* has become a serious clinical concern since the 1980s (1). During the past two decades, the rates of resistance to penicillin, other beta-lactams, and non-beta-lactam agents have been increasing rapidly in many parts of the world. In particular, data on rates of pneumococcal resistance from Asian countries at the end of the 1990s were alarming. International surveillance studies conducted in 1996-1997 (29) and 1998-1999 (18) in 11 Asian countries by the Asian Network for Surveillance of Resistant Pathogens (ANSORP) documented very high prevalence rates of penicillin and erythromycin resistance among *S. pneumoniae* clinical isolates and nasal carriage isolates from Korea, Japan, Vietnam, Thailand, Taiwan, and Sri Lanka, as well as the introduction and the spread of the Spanish 23F penicillin-resistant clone in Asia. The increasing prevalence of antimicrobial resistance among *S. pneumoniae* isolates is associated with an increasing incidence of invasive pneumococcal diseases in children (23), as well as clinical failures of antimicrobial treatment (6, 17, 19).

The present report describes the results of the third project of surveillance for pneumococcal resistance among clinical *S. pneumoniae* isolates collected from 14 centers in 11 countries in Asia and the Middle East between 2000 and 2001 by the ANSORP Study Group.

* Corresponding author. Mailing address: Division of Infectious Diseases, Samsung Medical Center, Sungkyunkwan University School of Medicine, Asian-Pacific Research Foundation for Infectious Diseases (ARFID), 50 Il-won dong, Kangnam-ku, Seoul 135-710, Korea. Phone: 82-2-3410-0320. Fax: 82-2-3410-0328. E-mail: jhsong@smc.samsung.co.kr or jhsong@ansorp.org.

MATERIALS AND METHODS

The 14 study centers in 11 countries in Asia and the Middle East that make up the ANSORP Study Group are listed in Acknowledgments. Clinical *S. pneumoniae* isolates were prospectively collected from patients with community-acquired pneumococcal diseases at the 14 study centers from January 2000 to June 2001. With the exception of lower respiratory tract specimens, all isolates

TABLE 1. Susceptibilities to beta-lactams and erythromycin of pneumococcal isolates from 12 Asian countries[a]

Country	No. of isolates	Penicillin				Amoxicillin-clavulanate				Cefuroxime				Ceftriaxone				Erythromycin			
		MIC (mg/liter)		% I	% R	MIC (mg/liter)		% I	% R	MIC (mg/liter)		% I	% R	MIC (mg/liter)		% I	% R	MIC (mg/liter)		% I	% R
		90%	Range			90%	Range			90%	Range			90%	Range			90%	Range		
Korea	31	4	<0.03–4	9.7	54.8	4	<0.03–8	6.5	9.7	8	<0.03–16	3.2	61.3	1	<0.25–4	3.2	3.2	>32	0.03–>32	0.0	80.6
China	111	2	<0.03–8	19.8	23.4	2	<0.03–32	2.7	7.3	4	<0.03–>32	4.5	19.8	1	<0.015–16	0.0	1.8	>32	<0.015–>32	0.9	73.9
Thailand	52	2	<0.03–4	26.9	26.9	2	<0.03–2	0.0	0.0	8	<0.03–8	1.9	36.5	1	<0.015–2	1.9	0.0	16	<0.015–>32	5.8	36.5
Taiwan	57	4	<0.03–8	24.6	38.6	2	<0.03–16	3.5	1.8	8	<0.03–8	8.8	40.4	1	0.03–2	1.8	0.0	>32	<0.015–>32	1.8	86.0
India	77	0.03	<0.03–1	7.8	0.0	<0.03	<0.03–0.5	0.0	0.0	0.12	<0.03–8	0.0	1.3	<0.25	<0.015–0.5	0.0	0.0	0.12	<0.015–4	0.0	1.3
Sri Lanka	42	2	<0.03–2	71.4	14.3	2	<0.03–2	0.0	0.0	4	<0.03–8	7.1	19.0	0.5	<0.25–1	0.0	0.0	8	0.015–>32	2.4	16.7
Singapore	35	2	<0.03–4	28.6	17.1	1	<0.03–2	0.0	0.0	4	<0.03–8	5.7	28.6	1	<0.015–1	0.0	0.0	>32	<0.015–>32	2.9	40.0
Malaysia	44	2	<0.03–4	9.1	29.5	2	<0.03–4	2.3	0.0	4	<0.03–8	2.3	29.5	1	<0.25–16	0.0	2.3	>32	0.015–>32	6.8	34.1
Vietnam	64	4	0.03–8	20.6	71.4	8	<0.03–16	14.3	22.2	8	<0.03–32	4.8	74.2	2	<0.015–4	9.5	3.2	>32	0.03–>32	1.6	92.1
Philippines	22	0.25	<0.03–0.25	21.3	0.0	0.03	<0.03–0.06	0.0	0.0	0.25	<0.03–0.5	0.0	0.0	0.25	0.12–0.5	0.0	0.0	16	<0.015–16	4.5	18.2
Saudi Arabia	39	1	<0.03–2	20.5	10.3	0.25	<0.03–2	0.0	0.0	4	<0.03–16	2.6	12.8	0.5	<0.25–1	0.0	0.0	0.12	<0.015–32	0.0	10.3
Hong Kong	112	4	<0.03–4	25.3	43.2	2	<0.03–16	0.9	3.6	8	<0.03–16	10.0	50.0	1	<0.015–2	3.7	0.0	>32	0.03–>32	0.0	76.8
Total	685	4	<0.03–8	23	29.4	2	<0.03–32	2.6	4.4	8	<0.03–>32	4.8	32.4	1	<0.015–16	1.9	0.9	>16	<0.015–>16	1.8	53.1

[a] Breakpoints for I and R are according to the NCCLS guidelines.

were recovered from clinical specimens representative of normally sterile body sites, such as blood, cerebrospinal fluid, ascitic fluid, pleural fluid, synovial fluid, sinus aspirates, and middle ear aspirates. Pneumococcal isolates from lower respiratory tract specimens were included only if *S. pneumoniae* was cultured from adequate respiratory specimens from patients with clinical and radiographic findings of pneumonia. Pneumococcal isolates from throat swab, nasal swab, or nasopharyngeal aspirate specimens were excluded from the study. Nosocomial infections caused by *S. pneumoniae*, which was defined as an infection occurring 3 days after admission to a hospital, were not included in the study. Isolates from study centers were transported to a central laboratory (Samsung Medical Center, Seoul, Korea) in a transport tube containing 12 ml of semisolid Ames transport medium with charcoal (Becton Dickinson, Sparks, Md.). Following storage at −70°C, the isolates were thawed and subcultured onto blood agar twice before susceptibility testing was performed.

In vitro susceptibility testing was performed by the broth microdilution test according to guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) (25). Fourteen antimicrobial agents were tested: penicillin, amoxicillin-clavulanate, cefuroxime, ceftriaxone, erythromycin, azithromycin, clarithromycin, clindamycin, ciprofloxacin, levofloxacin, moxifloxacin, gatifloxacin, doxycycline, and trimethoprim-sulfamethoxazole. Interpretive criteria for susceptibility were those indicated in NCCLS document M100-S13 (26). We used two separate interpretive breakpoints for meningeal and nonmeningeal *S. pneumoniae* isolates to define ceftriaxone resistance: MICs of ≥2 and ≥4 mg/liter, respectively. For the purpose of the study, ciprofloxacin MICs of ≥4 mg/liter were used to define the resistance category. *S. pneumoniae* ATCC 49619 was used as a control strain in each set of tests. Multidrug-resistant (MDR) *S. pneumoniae* was defined as a strain resistant to at least three of the antibiotic classes tested.

All isolates were serotyped or serogrouped by the capsular Quellung method with commercial antisera (Statens Seruminstitut, Copenhagen, Denmark), as recommended by the manufacturer. Serotyping was performed only for serogroups 23, 19, and 6.

Sixty-eight randomly selected serotype 19A, 19F, and 23F pneumococcal isolates were subjected to multilocus sequence typing (MLST) analysis. For MLST analysis, fragments of the *aroE*, *gdh*, *gki*, *recP*, *spi*, *xpt*, and *ddl* genes were obtained from chromosomal DNA by PCR and were directly sequenced with the primers described previously (4). Allele profiles are shown as a series of seven integers corresponding to the alleles at each of the loci, in the order *aroE*, *gdh*, *gki*, *recP*, *spi*, *xpt*, and *ddl*. The allele profiles determined were compared with data from the MLST website (www.mlst.net) and those for Pneumococcal Molecular Epidemiology Network clones (www.pneumo.com) (21). Isolates sharing more than five identical loci were defined to be the same clonal complex (4, 7). The BURST program was used to categorize the isolates into clonal complexes.

To analyze the risk factors for acquiring antibiotic-resistant *S. pneumoniae*, epidemiologic and clinical information was obtained at the time of patient enrollment the in the study. These factors included baseline demographic data (age and sex), clinical information (antibiotic use within the previous 3 months and underlying conditions), and others (serotype, specimen source, and resistance to other antibiotics). Univariate and multivariate logistic regression analyses were performed to evaluate the risk factors for acquiring antibiotic-resistant pneumococci.

RESULTS

A total of 685 isolates of *S. pneumoniae* which caused various pneumococcal diseases were collected from 14 centers from January 2000 to June 2001. The number of isolates varied by center, according to the duration of collection (Table 1). Of these, 243 (35.5%) were isolated from lower respiratory tract specimens, 208 (30.4%) were isolated from blood cultures, 65 (9.5%) were isolated from middle ear aspirates, 56 (8.2%) were isolated from cerebrospinal fluid, and 113 (16.5%) were isolated from other sites. Of the 632 isolates for which the ages of the patients were available, 264 (41.8%) were from patients ≤5 years old, 54 (8.6%) were from patients between 5 and 15 years old, and 313 (49.6%) were from patients >15 years old.

The results of in vitro susceptibility testing for penicillin and beta-lactam agents are presented in Table 1. Of the 685 *S. pneumoniae* isolates from Asia, 483 (52.4%) were not suscep-

TABLE 2. Changing trend of penicillin nonsusceptibility among pneumococcal isolates from Asia

Country	MIC_{90} (mg/liter)			% Nonsusceptible strains (I/R)		
	Clinical pathogens (n = 685) (2000–2001)	Carriage isolates[a] (n = 1,105) (1998–1999)	Clinical isolates[b] (n = 996) (1996–1997)	Clinical pathogens (n = 685) (2000–2001)	Carriage isolates[a] (n = 1,105) (1998–1999)	Clinical isolates[b] (n = 996) (1996–1997)
Korea	4	2	8	9.7/54.8	54.5/31.3	24.3/55.4
China	2	0.06	0.06	19.8/23.4	13.4/0	9.8/0
Thailand	2	0.75	1	26.9/26.9	41.8/3.8	35.7/22.2
Taiwan	4	1	8	24.6/38.6	43.5/47.8	9.3/29.4
India	0.03	0.06	0.06	7.8/0	12.8/0	3.8/0
Sri Lanka	2	0.9	0.5	71.4/14.3	70.6/5.9	41.2/0
Singapore	2	2	2	28.6/17.1	26.8/19.5	4.9/18.2
Malaysia	2	0.06	0.06	9.1/29.5	8.4/4.9	6.0/3.0
Vietnam	4	1.5	NA[c]	20.6/71.4	58.2/12.2	28.2/32.6
Philippines	0.25	0.02	NA	27.3/0	2.1/0	NA/NA
Saudi Arabia	2	1.5	NA	20.5/10.3	34.6/15.4	NA/NA
Hong Kong	4	NA	NA	24.1/43.8	NA/NA	NA/NA
Total	2.36	0.9	2.46	24.2/27.5	33.3/12.8	18.1/17.8

[a] Data are from reference 18.
[b] Data are from reference 29.
[c] NA, not available.

tible to penicillin; 23% were penicillin intermediate (I; MICs = 0.12 to 1 mg/liter), and 29.4% were penicillin resistant (R; MICs ≥ 2 mg/liter) (Table 1). Prevalence rates of penicillin nonsusceptibility varied widely by country, ranging from 7.8% (Vellore, India) to 92% (Ho Chi Minh City, Vietnam). Isolates from Vietnam showed the highest prevalence of penicillin nonsusceptibility (I, 20.6%; R, 71.4%) followed by those from Sri Lanka (I, 71.4%; R, 14.3%), Hong Kong (I, 25.3%; R, 43.2%), Korea (I, 9.7%; R, 54.8%), Taiwan (I, 24.6%; R, 38.6%), and Thailand (I, 26.9%; R, 26.9%). The penicillin MIC at which 90% of isolates are inhibited (MIC_{90}) was 4 mg/liter among isolates from Vietnam, Hong Kong, Korea, and Taiwan, while the MIC_{90}s for isolates from India and the Philippines were very low (0.03 and 0.25 mg/liter, respectively). If we compare the prevalence rate of penicillin resistance among clinical pathogens from this study with those among clinical isolates (1996-1997) and carriage isolates (1998-1999) from previous ANSORP studies, the rate of penicillin resistance has markedly increased among isolates from Vietnam, China, Sri Lanka, and Malaysia, while isolates from Korea, Taiwan, and Thailand showed persistently high rates of penicillin resistance (Table 2). The level of penicillin resistance has also increased, especially among isolates from Vietnam, Hong Kong, Korea, and Taiwan, as indicated by MIC_{90}s of 4 mg/liter. Penicillin MICs ≥4 mg/liter were found for 36.5% of pneumococcal isolates from Vietnam, 25.8% of isolates from Korea, and 14.3% of isolates from Hong Kong. The highest penicillin MIC was 8 mg/liter for isolates from Taiwan (three isolates), China (one isolate), and Vietnam (one isolate). All cases caused by these highly resistant strains were successfully treated with various antimicrobial therapies. The rate of penicillin nonsusceptibility was significantly higher in patients ≤5 years of age (I, 23.1%; R, 37.5%) than patients >5 years of age (I, 23.1%; R, 26.6%) (P = 0.005). Pneumococcal isolates resistant to penicillin were also resistant to other antimicrobial agents. Penicillin-resistant isolates were resistant to amoxicillin-clavulanate (13.9%), cefuroxime (93.1%), ceftriaxone (7.9%), erythromycin (92.6%), trimethoprim-sulfamethoxazole (89.1%), clindamycin (54.5%), and ciprofloxacin (8.9%). Multivariate analyses showed that age ≤5 years (odds ratio [OR], 1.7; 95% confidence interval [CI], 1.2 to 2.4; P = 0.002), underlying pulmonary diseases (OR, 2.0; 95% CI, 1.3 to 3.1; P = 0.003), malignancy (OR, 2.3; 95% CI, 1.2 to 4.6; P = 0.015), and steroid use (OR, 2.8; 95% CI, 1.1 to 7.4; P = 0.032) were independent risk factors for penicillin resistance among pneumococcal isolates. The overall rate of amoxicillin-clavulanate resistance (I and R) was 7.9%; only 4.4% of isolates were resistant. The rates of ceftriaxone resistance were 0.9% among nonmeningeal isolates (MICs ≥ 4 mg/liter) and 4.1% among meningeal isolates (MICs ≥ 2 mg/liter). Although the prevalence rates of amoxicillin and ceftriaxone resistance were not high, we found strains highly resistant to amoxicillin (MICs ≥ 8 mg/liter) and/or extended-spectrum cephalosporins (ceftriaxone MICs ≥ 8 mg/liter) among isolates from Vietnam (n = 13), China (n = 6), Hong Kong (n = 3), Korea (n = 3), Taiwan (n = 1), and Malaysia (n = 1).

A total of 376 (54.9%) isolates were not susceptible to erythromycin; 1.8% were intermediate, and 53.1% were resistant. Very high rates of erythromycin resistance (MICs ≥ 1 mg/liter) were observed in Vietnam (92.1%), Taiwan (86%), Korea (80.6%), Hong Kong (76.8%), and China (73.9%). On the other hand, erythromycin resistance was relatively not prevalent in India (1.3%), Saudi Arabia (10.3%), and Sri Lanka (16.7%). Of the erythromycin-resistant isolates, 217 (59.6%) were clindamycin resistant and had the macrolide-lincosamide-streptrogramin B (MLS_B) phenotype. The MIC_{90}s of erythromycin were >32 mg/liter for isolates from Korea, Vietnam, China, Taiwan, Singapore, Malaysia, and Hong Kong. Most of penicillin-resistant isolates (92.6%) were resistant to erythromycin, while 23.9% of penicillin-susceptible isolates were also resistant to erythromycin. The rate of erythromycin resistance was significantly higher among isolates from patients younger than age 5 years (OR, 3.1; 95% CI, 1.9 to 5.2; P = 0.001) and among isolates with penicillin resistance (OR, 15.4; 95% CI, 9.5 to 25; P = 0.001).

Forty-one (6%) isolates were resistant to ciprofloxacin (MICs ≥ 4 mg/liter) (Table 3). Isolates from Hong Kong

TABLE 3. Susceptibilities to fluoroquinolones of pneumococcal isolates from 12 Asian countries[a]

Country	Levofloxacin				Moxifloxacin				Gatifloxacin				Ciprofloxacin		
	MIC (mg/liter)		% I	% R	MIC (mg/liter)		% I	% R	MIC (mg/liter)		% I	% R	MIC (mg/liter)		% R
	90%	Range			90%	Range			90%	Range			90%	Range	
Korea	2	0.25–2	0.0	0.0	0.25	0.06–0.5	0.0	0.0	0.5	0.12–1	0.0	0.0	2	0.25–4	6.5
China	1	0.12–2	0.0	0.0	0.25	0.004–1	0.0	0.0	0.5	<0.03–1	0.0	0.0	2	0.12–>32	3.6
Thailand	1	0.5–2	0.0	0.0	0.25	0.03–0.5	0.0	0.0	0.5	<0.03–1	0.0	0.0	2	0.5–8	3.8
Taiwan	1	0.5–2	0.0	1.8	0.25	0.03–0.5	1.8	0.0	0.5	0.03–1	1.8	1.8	2	0.5–8	7
India	1	0.06–8	0.0	1.3	0.25	0.015–2	1.3	0.0	0.5	<0.03–8	0.0	1.4	2	0.12–16	4
Sri Lanka	1	0.5–2	0.0	0.0	0.25	0.06–0.5	0.0	0.0	0.5	0.12–1	0.0	0.0	2	0.5–4	9.5
Singapore	1	0.12–4	2.9	0.0	0.12	0.015–0.25	0.0	0.0	0.5	0.06–1	0.0	0.0	2	0.5–16	5.9
Malaysia	1	0.015–2	0.0	0.0	0.25	<0.002–1	0.0	0.0	0.5	0.06–1	0.0	0.0	2	<0.015–4	4.6
Vietnam	1	0.25–1	0.0	0.0	0.25	0.06–0.25	0.0	0.0	0.5	0.06–1	0.0	0.0	2	0.12–>4	4.8
Philippines	1	0.5–2	0.0	0.0	0.25	0.03–0.5	0.0	0.0	0.5	0.06–1	0.0	0.0	2	0.5–4	9.1
Saudi Arabia	1	<0.004–2	0.0	0.0	0.25	<0.002–0.25	0.0	0.0	0.5	0.015–1	0.0	0.0	2	<0.015–4	2.6
Hong Kong	2	0.015–32	0.0	8.0	0.5	0.015–4	6.3	1.8	1	0.03–8	0.9	8.3	4	0.015–32	11.8
Total	1	<0.004–32	0.1	1.6	0.25	<0.002–4	1.3	0.3	0.5	0.03–8	0.3	1.6	2	0.008–>32	6

[a] MIC breakpoints for individual fluoroquinolones are as follows: for levofloxacin, I = 4 mg/liter and R = 8 mg/liter; for moxifloxacin, I = 2 mg/liter and R = 4 mg/liter; for gatifloxacin, I = 2 mg/liter and R ≥ 4 mg/liter; and for ciprofloxacin R ≥ 4 mg/liter.

showed the highest rate of ciprofloxacin resistance (11.8%), followed by isolates from Sri Lanka (9.5%), the Philippines (9.1%), and Korea (6.5%). Isolates from Hong Kong also showed higher rates of resistance to levofloxacin (8%), gatifloxacin (8.3%), and moxifloxacin (1.8%) than isolates from other Asian countries, where the prevalence of resistance to these newer fluoroquinolones was extremely low at the time of the study.

The overall rate of MDR *S. pneumoniae*, which was defined as resistance to at least three classes of antibiotics, was 26.8%, with 71.4% of the isolates from Vietnam, 44.9% of the isolates from Hong Kong, 30.9% of the isolates from Taiwan, and 45.2% of the isolates from Korea being MDR. The most common pattern of MDR was resistance to penicillin, erythromycin, and trimethoprim-sulfamethoxazole.

The most prevalent serogroups among the clinical pathogens from Asia were 19, 23, 6, 14, and 9, which accounted for 65.6% of all isolates (Table 4). Of the 271 isolates from children younger than age 5 years, serotypes 19F (21.8%), 23F (14.7%), 14 (10%), 6B (6.6%), 9 (4.5%), 6A (3.2%), and 19A (3%) were the most common ones. MLST analysis showed that most of the isolates belonged to either the Taiwan^{19F} clone (30 isolates) or the Spain^{23F} clone (33 isolates) (Table 5).

DISCUSSION

The results of this study indicate that the rates of antimicrobial resistance among *S. pneumoniae* isolates in Asia continue to increase. Since this study was a continuation of multinational surveillance projects that included two previous ANSORP studies, it could provide a unique opportunity to investigate the changing trend in resistance rates in Asia over a 5-year period. Previous ANSORP studies with clinical isolates (1996-1997) (29) and nasal carriage isolates (1998-1999) (18) have already revealed that many Asian countries had serious problems with in vitro resistance to penicillin and non-beta-lactam agents among *S. pneumoniae* isolates. Although direct comparison may not be possible due to the different characteristics of the isolates, resistance rates among pneumococcal isolates in this study strongly suggest a substantial increase in

TABLE 4. Serotype distributions of pneumococcal isolates in 11 Asian countries

Country	No. of isolates (adults/children)	% of strains by serotype (adults/children)												
		19				23		6		14	9	4	18	Others
		A	B	C	F	A	F	A	B					
Korea	27/4	3.2/0.0		3.2/0.0	19.4/6.5		3.2/3.2	3.2/0.0	6.5/3.2	3.2/0.0	6.5/0.0	3.2/0.0		35.5/0.0
China	13/96	0.9/3.7			1.8/18.3	0.0/1.8	0.9/7.4	0.0/5.5		0.9/7.4	0.9/3.7		0.0/5.5	6.4/34.9
Thailand	34/17	1.9/1.9			9.6/5.8		5.8/7.7	7.7/1.9	0.0/1.9	1.9/3.8	1.9/3.8		5.8/1.9	32.8/3.9
Taiwan	31/21				11.5/3.8	3.8/0.0	9.6/5.8		3.8/7.7	5.8/17.3	5.8/1.9			19.4/3.8
India	57/16	4.1/0.0	1.4/0.0		4.1/1.4	2.7/4.1	2.7/4.1	2.7/0.0	1.4/2.7	4.1/2.7	1.4/2.7	1.4/1.4	2.7/0.0	49.4/5.5
Sri Lanka	40/2	2.4/0.0			31.0/0.0		23.8/0.0		4.8/0.0	14.2/2.4	9.5/0.0			9.5/2.4
Singapore	10/6				0.0/12.5		18.8/12.5		0.0/6.2	12.5/6.2				31.3/0.0
Malaysia	27/14	0.0/4.9			24.4/9.8	2.4/0.0	7.3/0.0	4.9/4.9	4.9/0.0	0.0/2.4		2.4/0.0	0.0/4.9	19.5/7.3
Vietnam	6/57				3.2–38.0		1.6/25.4	1.6/0.0	0.0/1.6	3.2/14.3	0.0/1.6			0.0/9.5
Philippines	4/18	4.5/4.5			0.0/13.6				0.0/18.2		0.0/9.1	4.5/0.0		9.1/36.5
Hong Kong	97/14	0.9/0.0			21.6/4.5		18.9/2.7	2.7/0.0	6.3/3.6	5.4/1.8		0.9/0.0	0.9/0.0	29.8/0.0
Total	346/265	1.6/1.4	0.1/0.0	0.3/0.0	11.5/10.3	0.8/0.3	8.4/6.3	2.1/1.1	2.5/4.1	4.7/5.3	2.4/2.1	1.1/0.1	0.9/1.1	22.0/9.5

TABLE 5. Results of MLST analysis

Country[a]	No. of isolates					
	Serotype			Clonal complex[b]		
	19A	19F	23F	Taiwan^{19F}	Spain^{23F}	Others
Korea (8)	1	5	2	6	1	1
China (9)	1	5	3	4	2	3
Thailand (5)		2	3		5	
Taiwan (7)		3	4	4	3	
Sri Lanka (2)			2		1	1
Singapore (3)		1	2		3	
Malaysia (9)		9		5	4	
Vietnam (11)		5	6	5	6	
Hong Kong (14)		7	7	6	8	
Total (68)	2	37	29	30	33	5

[a] The number of isolates used in MLST analysis is shown in parentheses.
[b] Clonal complex as defined by McGee et al. (21) and the Pneumococcal Molecular Epidemiology Network (www.pneumo.com).

antimicrobial resistance among *S. pneumoniae* isolates in the region. Resistance rates among study centers in each country varied widely. For example, the rates of penicillin resistance (MICs $\geq$ 2 mg/liter) ranged from 0% (India and Philippines) to 71.4% (Vietnam), and the rates of resistance to non-beta-lactam agents varied in a similar manner.

The increasing prevalence of penicillin resistance in Asia was consistent with recent reports on the emergence of penicillin resistance in Hong Kong (15), Taiwan (12), and Vietnam (27). The most remarkable finding with regard to the penicillin resistance in this study is an incredibly high prevalence of penicillin resistance in Vietnam (Ho Chi Minh City). In a university hospital in Ho Chi Minh City, 92% of clinical pathogens were not susceptible to penicillin and 71% of the isolates were fully resistant to penicillin, with penicillin MICs of $\geq$2 mg/liter. This is a very alarming increase in high-level penicillin resistance within the past 5 years. Previous ANSORP studies showed that 32.6% of clinical isolates in 1996-1997 and 12.2% of carriage isolates in 1998-1999 were fully resistant to penicillin in a university hospital in Ho Chi Minh City. Although these data were obtained from one university hospital and are for a relatively small number of isolates, the data strongly suggest the rapid surge of penicillin resistance among pneumococcal isolates in that area. Another important observation in this study is a rapid increase in penicillin resistance in China (Beijing and Shanghai) and Malaysia (Kuala Lumpur). The overall rates of penicillin nonsusceptibility in Beijing and Shanghai, China, were about 10% (mostly intermediate) in the previous studies in 1996-1999 (18, 29), but the rate has increased to 43% (rate of resistance, 23.4%) in this study. The same trend was noted among isolates from Kuala Lumpur, Malaysia.

Not only the proportion of nonsusceptible strains but also the level of penicillin resistance have continued to increase in many Asian countries. In Vietnam, Hong Kong, Korea, Taiwan, China, and Malaysia, penicillin-resistant strains were more common than penicillin-intermediate strains. In this study, we found five strains (three from Taiwan and one each from China and Vietnam) for which penicillin MICs were 8 mg/liter. Recently, pneumococci with high-level resistance to amoxicillin (MICs $\geq$ 4 mg/liter) and/or extended-spectrum cephalosporins (cefotaxime MICs $\geq$ 4 mg/liter) have been identified in France (3) and Canada (20). We found 27 strains (13 from Vietnam, 6 from China, 3 from Korea, 3 from Hong Kong, and 1 each from Malaysia and Taiwan) for which amoxicillin MICs were $\geq$8 mg/liter and/or ceftriaxone MICs were $\geq$8 mg/liter. The emergence of such strains is of particular concern in the treatment of pneumococcal meningitis, because ceftriaxone is an important component of the combination regimens for pneumococcal meningitis.

As documented in previous reports (16), children younger than age 5 years were more frequently infected with penicillin-resistant strains. Frequent exposures to antimicrobial agents and day care center attendance would be important risk factors for the higher prevalence of penicillin resistance among isolates from children (22).

The remarkable increases in the prevalence and the level of macrolide resistance among clinical pathogens from Asia are of particular concern. Reported rates of erythromycin resistance in the Western Hemisphere were high in France (58.1%), Spain (57.1%), and the eastern south-central parts of the United States (47%) (8, 31; D. Felmingham and R. N. Gruneberg, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1790, 2000). However, macrolide resistance was reported to be more prevalent in Asian countries than in Western countries. Reports from Hong Kong and Taiwan showed that 80 to 91% of pneumococcal isolates were resistant to erythromycin (13, 14). According to the data from the present study, the prevalence rates of macrolide resistance were alarmingly high in Vietnam, Taiwan, South Korea, Hong Kong, and China, where more than 70% of clinical pathogens were fully resistant to erythromycin. Erythromycin resistance in pneumococci is due to the modification of the drug-binding site [which is regulated by the *erm*(B) gene], which is usually associated with the MLS_B phenotype and high-level resistance to erythromycin, with MICs of $\geq$64 mg/liter. Low-level erythromycin resistance, with MICs of 1 to 32 mg/liter, is due to the active efflux of the drug [which is regulated by the *mef*(A) gene] (5). The efflux mechanism caused by the *mef*(A) gene is predominant in macrolide-resistant pneumococci in North America (28), while *erm*(B)-mediated ribosomal methylation has been found in >80% of erythromycin-resistant *S. pneumoniae* isolates in most European countries (9). On the basis of the distributions of the MICs for erythromycin-resistant strains and the rate of resistance to clindamycin in this study, the MLS_B phenotype is predominant in Korea, China, Taiwan, Vietnam, and Hong Kong. Molecular characterization of macrolide-resistant strains from Asia showed that the *erm*(B) gene was found in more than 50% of pneumococcal isolates either singly or dually with the *mef*(A) gene in Korea, China, Vietnam, and Taiwan (30). Recently, clinical failures of macrolide treatment for pneumococcal infections caused by macrolide-resistant strains have been reported (19, 24). Given the widespread emergence of high-level resistance to erythromycin among pneumococci in Asia, as documented in this study, the use of a single macrolide for the treatment of pneumococcal diseases may result in the clinical failure of antimicrobial therapy.

Data from this study also showed the present situation of fluoroquinolone resistance among pneumococcal isolates in

Asia. Recently, Hong Kong investigators demonstrated the high rates of resistance of *S. pneumoniae* isolates to various fluoroquinolones (10) and the presence of the Spanish 23F clone, which has acquired fluoroquinolone resistance while circulating in Hong Kong (11). Our data confirmed a higher rate of ciprofloxacin resistance among isolates from Hong Kong than among isolates from other Asian countries. Although the overall rates resistance to fluoroquinolones remain low in most Asian countries, we found 12 strains of diverse serotypes (7 from Hong Kong and 1 each from China, Taiwan, India, Singapore, and Thailand) with high-level resistance to ciprofloxacin (MICs $\geq$ 8 mg/liter). These strains were also resistant to newer fluoroquinolones, such as moxifloxacin and gatifloxacin. The emergence of these strains highly resistant to fluoroquinolones will be a concern in the future with regard to the treatment of pneumococcal pneumonia. Clinical failures of levofloxacin for the treatment of pneumococcal pneumonia have already been documented (2).

The serotype distributions among the clinical pathogens from Asia showed that major serogroups were 19 (19F), 23 (23F), 6 (6B), 14, and 9, which are contained in the seven-valent conjugate vaccine. Given the high prevalence of antibiotic-resistant *S. pneumoniae* in Asia, pneumococcal vaccination should be applied more widely in Asia.

The MLST analysis performed in this study showed that the spread of specific resistant clones, such as the Taiwan^{19F} clone and the Spain^{23F} clone, in Asia could be one of the major reasons for the rapid increases in penicillin and macrolide resistance as well as MDR in *S. pneumoniae*.

There may be some limitations in interpreting the data from this study. Since pneumococcal isolates were collected from one or two referral centers, mainly in urban areas of each country, and the number of isolates was relatively few in some centers, data from this study may not reflect the overall resistance status in a whole country. On the basis of the information from this study, further surveillance of pneumococcal resistance with more isolates from more centers is strongly warranted, especially in countries where resistance rates have markedly increased. Also, we could not identify the direct relationship between the amounts of antibiotics used or the history of previous antibiotic use and the emergence of resistance due to a lack of relevant information in many countries.

In conclusion, this multinational surveillance study conducted by ANSORP clearly documents distinctive increases in the prevalence rates and the levels of penicillin and macrolide resistance in many Asian countries, which are among the highest in the world that have been published to date, as well as the emergence of fluoroquinolone resistance in Hong Kong. The injudicious use of antibiotics and the clonal spread of resistant strains in Asia could be the major reasons for the rapid increases in the rates of pneumococcal resistance in Asia. Continuous surveillance of antimicrobial resistance among pneumococcal isolates as well as appropriate use of antibiotics and pneumococcal vaccination is critically required in Asia.

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REFERENCES

1. **Adam, D.** 2002. Global antibiotic resistance in *Streptococcus pneumoniae*. J. Antimicrob. Chemother. **50**(Topic T1)**:**1–5.
2. **Davidson, R., R. Cavalcanti, J. L. Brunton, D. J. Bast, J. C. S. de Azavedo, P. Kibsey, C. Fleming, and D. E. Low.** 2002. Resistance to levofloxacin and failure of treatment of pneumococcal pneumonia. N. Engl. J. Med. **346:**747–750.
3. **Doit, C. C. Loukil, F. Fitoussi, P. Geslin, and E. Bingen.** 1999. Emergence in France of multiple clones of clinical *Streptococcus pneumoniae* isolates with high-level resistance to amoxicillin. Antimicrob. Agents Chemother. **43:**1480–1483.
4. **Enright, M. C., and B. G. Spratt.** 1998. A multilocus sequence typing scheme for *Streptococcus pneumoniae*: identification of clones associated with serious invasive disease. Microbiology **144:**3049–3069.
5. **Farrell, D. J., I. Morrissey, S. Bakker, and D. Felmingham.** 2002. Molecular characterization of macrolide resistance mechanisms among *Streptococcus pneumoniae* and *Streptococcus pyogenes* isolated from the PROTEKT 1999–2000 study. J. Antimicrob. Chemother. **50:**39–47.
6. **Feikin, D. R., A. Schuchat, M., Kolczak, N. L. Barrett, L. H. Harrison, L. Lefkowitz, A. McGeer, M. M. Farley, D. J. Vugia, C. Lexau, K. R. Stefonek, J. E. Patterson, and J. H. Jorgensen.** 2000. Mortality from invasive pneumococcal pneumonia in the era of antibiotic resistance, 1995–1997. Am. J. Public Health **90:**223–229.
7. **Feil, E. J., J. E. Cooper, J. Grundmann, D. A. Robinson, M. C. Enright, T. Berendt, S. J. Peacock, J. Maynard Smith, M. Murphy, B. G. Spratt, C. E. Moore, and N. P. J. Day.** 2003. How clonal is *Staphylococcus aureus*? J. Bacteriol. **185:**3307–3316.
8. **Fenoll, A., C. M. Bourgon, R. Munoz, D. Vicioso, and J. Casal.** 1991. Serotype distribution and antimicrobial resistance of *Streptococcus pneumoniae* isolates causing systemic infections in Spain, 1979–1989. Rev. Infect. Dis. **13:**56–60.
9. **Fitoussi, F., C. Doit, P. Geslin, N. Brahimi, and E. Bingen.** 2001. Mechanisms of macrolide resistance in clinical pneumococcal isolates in France. Antimicrob. Agents Chemother. **45:**636–638.
10. **Ho, P. L., R. W. Yung, D. N. Tsang, T. L. Que, M. Ho, W. H. Seto, T. K. Ng, W. C. Yam, and W. W. Ng.** 2001. Increasing resistance of *Streptococcus pneumoniae* to fluoroquinolones: results of a Hong Kong multicentre study in 2000. J. Antimicrob. Chemother. **48:**659–665.
11. **Ho, P. L., W. C. Yam, T. K. Cheung, W. W. Ng, T. L. Que, D. N. Tsang, T. K. Ng, and W. H. Seto.** 2001. Fluoroquinolone resistance among *Streptococcus pneumoniae* in Hong Kong linked to the Spanish 23F clone. Emerg. Infect. Dis. **7:**906–908.
12. **Hsueh, P.-R., L.-J. Teng, L.-N. Lee, P.-C. Yang, S.-W. Ho, and K.-T. Luh.** 1999. Extremely high incidence of macrolide and trimethoprim-sulfamethoxazole resistance among clinical isolates of *Streptococcus pneumoniae* in Taiwan. J. Clin. Microbiol. **37:**897–901.
13. **Hsueh, P. R., L. J. Teng, L. N. Lee, P. C. Yang, S. W. Ho, H. C. Lue, and K. T. Luh.** 2002. Increased prevalence of erythromycin resistance in streptococci: substantial upsurge in erythromycin-resistant M phenotype in *Streptococcus pyogenes* (1979–1998) but not in *Streptococcus pneumoniae* (1985–1999) in Taiwan. Microb. Drug Resist. **8:**27–33.
14. **Ip, M., D. J. Lyon, R. W. Yung, C. Chan, and A. F. Cheng.** 2001. Macrolide resistance in *Streptococcus pneumoniae* in Hong Kong. Antimicrob. Agents Chemother. **45:**1578–1580.
15. **Ip, M., D. J. Lyon, R. W. H. Yung, L. Tsang, and A. F. B. Cheng.** 2002. Introduction of new clones of penicillin-nonsusceptible *Streptococcus pneumoniae* in Hong Kong. J. Clin. Microbiol. **40:**1522–1525.
16. **Kaplan, S. L., E. O. Mason, W. J. Barson, E. R. Wald, M. Arditi, T. Q. Tan, G. E. Schutze, J. S. Bradley, L. B. Givner, K. S. Kim, and R. Yogev.** 1998. Three-year multicenter surveillance of systemic pneumococcal infections in children. Pediatrics **102:**538–545.
17. **Kays, M. B., M. F. Wack, D. W. Smith, and G. A. Denys.** 2002. Azithromycin

treatment failure in community-acquired pneumonia caused by *Streptococcus pneumoniae* resistant to macrolides by a 23S rRNA mutation. Diagn. Microbiol. Infect. Dis. **43:**163–165.

18. **Lee, N. Y., J. H. Song, S. Kim, K. R. Peck, K. M. Ahn, S. I. Lee, Y. Yang, J. Li, A. Chongthaleong, S. Tiengrim, N. Aswapokee, T. Y. Lin, J. L. Wu, C. H. Chiu, M. K. Lalitha, K. Thomas, T. Cherian, J. Perera, T. T. Yee, F. Jamal, U. C. Warsa, P. H. Van, C. C. Carlos, A. M. Shibl, M. R. Jacobs, and P. C. Appelbaum.** 2001. Carriage of antibiotic-resistant pneumococci among Asian children: a multinational surveillance by the Asian Network for Surveillance of Resistant Pathogens (ANSORP). Clin. Infect. Dis. **32:**1463–1469.
19. **Lonks, J. R., J. Garay, and A. A. Medeiros.** 2002. Implications of antimicrobial resistance in the empirical treatment of community-acquired respiratory infections: the case of macrolides. J. Antimicrob. Chemother. **50**(Suppl. S2)**:**87–91.
20. **Low, D. E., J. de Azavedo, K. Weiss, T. Mazzulli, M. Kuhn, D. Church, K. Forward, G. Zhanel, A. Simor, and A. McGeer.** 2002. Antimicrobial resistance among clinical isolates of *Streptococcus pneumoniae* in Canada during 2000. Antimicrob. Agents Chemother. **46:**1295–1301.
21. **McGee, L., L. McDougal, J. Zhou, B. G. Spratt, F. C. Tenover, R. George, R. Hakenbeck, W. Hryniewicz, J. C. Lefévre, A. Tomasz, and K. P. Klugman.** 2001. Nomenclature of major antimicrobial-resistant clones of *Streptococcus pneumoniae* defined by the pneumococcal molecular epidemiology network. J. Clin. Microbiol. **39:**2565–2571.
22. **Melander, E., S. Mölstad, K. Persson, H. B. Hansson, M. Söderström, and K. Ekdahl.** 1999. Previous antibiotic consumption and other risk factors for carriage of penicillin-resistant *Streptococcus pneumoniae* in children. Eur. J. Clin. Microbiol. Infect. Dis. **17:**834–838.
23. **Morita, J. Y., E. R. Zell, R. Dalina, M. M. Farley, J. Hadler, L. H. Harrison, L. Lefkowitz, A. Reingold, B. A. Kupronis, A. Schuchat, and C. G. Whitney.** 2002. Association between antimicrobial resistance among pneumococcal isolates and burden of invasive pneumococcal disease in the community. Clin. Infect. Dis. **35:**420–427.
24. **Musher, D. M., M. E. Dowell, V. D. Shortridge, J. H. Jorgensen, and P. Le Magueres.** 2002. Emergence of macrolide resistance during treatment of pneumococcal pneumonia. N. Engl. J. Med. **346:**630–631.
25. **National Committee for Clinical Laboratory Standards.** 2003. Performance standards for antimicrobial susceptibility testing; 13th informational supplement, vol. 23, no. 1. M100-S13. National Committee for Clinical Laboratory Standards, Wayne, Pa.
26. **National Committee for Clinical Laboratory Standards.** 2003. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard M7-A6, 6th ed. National Committee for Clinical Laboratory Standards, Wayne, Pa.
27. **Parry, C. M., T. S. Diep, J. Waine, N. T. T. Hoa, M. Gainsborough, D. Nga, C. Davies, N. H. Phu, T. T. Hien, N. J. White, and J. J. Farrar.** 2000. Nasal carriage in Vietnamese children of *Streptococcus pneumoniae* resistant to multiple antimicrobial agents. Antimicrob. Agents Chemother. **44:**484–488.
28. **Shortridge, V. D., G. V. Doern, A. B. Brueggemann, J. M. Beyer, and R. K. Flamm.** 1999. Prevalence of macrolide resistance mechanisms in *Streptococcus pneumoniae* isolates from a multicenter antibiotic resistance surveillance study conducted in the United States in 1994–1995. Clin. Infect. Dis. **29:** 1186–1188.
29. **Song, J. H., N. Y. Lee, S. Ichiyama, R. Yoshida, Y. Hirakata, W. Fu, A. Chongthaleong, N. Aswapokee, C. H. Chiu, M. K. Lalitha, K. Thomas, J. Perera, T. T. Yee, F. Jamal, U. C. Warsa, B. X. Vinh, M. R. Jacobs, P. C. Appelbaum, and C. H. Pai.** 1999. Spread of drug-resistant *Streptococcus pneumoniae* in Asian countries: Asian Network for Surveillance of Resistant Pathogens (ANSORP) study. Clin. Infect. Dis. **28:**1206–1211.
30. **Song, J. H., H.-H. Chang, J. Y. Suh, K. S. Ko, S.-I. Jung, W. S. Oh, K. R. Peck, N. Y. Lee, Y. Yang, A. Chongthaleong, N. Aswapokee, C.-H. Chiu, M. K. Lalitha, J. Perera, T. T. Yee, G. Kumararansinghe, F. Jamal, A. Kamarulazaman, N. Parasakthi, P. H. Van, T. So, and T. K. Ng and on Behalf of the ANSORP Study Group.** 2004. Macrolide resistance and genotypic characterization of *Streptococcus pneumoniae* in Asian countries: a study of the Asian Network for Surveillance of Resistant Pathogens (ANSORP). J. Antimicrob. Chemother. **53:**457–463.
31. **Thornsberry, C., P. T. Ogilvie, H. P. Holley, and D. F. Sahm.** 1999. Survey of susceptibilities of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* isolates to 26 antimicrobial agents: a prospective U.S. study. Antimicrob. Agents Chemother. **43:**2612–2623.