National Antimicrobial Resistance Surveillance among Clinical Isolates of *Streptococcus pneumoniae* in Thailand

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As part of the continuing national antimicrobial surveillance, the national antimicrobial resistance surveillance thailand (NARST), data of all clinical isolates of Streptococcus pneumoniae were collected from 28 hospitals in Thailand from 2000 to 2005. Epidemiological and microbiological data were obtained and analyzed using the WHONET software program. Among all isolates tested for antimicrobial susceptibility, the rates of penicillin resistance were constantly high, ranging from 42.4% in 2000 to 47.7% in 2005. The third-generation cephalosporin resistance rate, determined by Epsilon test (E-test) in 10% to 15% of all isolates each year, ranged from 2.1% to 8.4%. The rates of erythromycin resistance ranged from 24.2% to 30.3%. Surprisingly, one isolate in 2005 was resistant to levofloxacin. The rates of multi-drug resistance ranged from 14.8% to 34.3%.

In conclusion, the present (NARST) study documents remarkable increase of penicillin, erythromycin, and multi-drug resistance rates in Thailand, especially among isolates from the North, the Center, the East, and Bangkok; from university hospitals; from young children; and from non-sterile specimens.

Keywords: *Anti-infective agents, Drug resistance, Bacterial, Epidemiology, Microbial sensitivity tests, Population surveillance, Prevalence, Streptococcus pneumoniae, Thailand*

J Med Assoc Thai 2009; 92 (Suppl 4): S19-33
Full text. e-Journal: *http://www.mat.or.th/journal*

Streptococcus pneumoniae is an important pathogen in many community-acquired respiratory infections including bacterial sinusitis, otitis media, and pneumonia as well as in invasive infections including meningitis and bacteremia. The first isolate of penicillin-resistant *S. pneumoniae* (MIC = 0.6 mg/mL) (PRSP) was reported from Australia in 1967[1]. PRSP isolates have been increasingly identified globally, particularly since the late 1980s[2-5]. The rates of penicillin resistance among *S. pneumoniae* are as high as 60% in some parts of Latin America and as high as 80% in some countries in Asia[6,7]. Furthermore, during the past two decades the rates of resistance to other antimicrobials including cephalosporins, macrolides, and fluoroquinolones have been increasingly recognized in many parts of the world[3-5,7].

The present study describes the results of the National Antimicrobial Resistance Surveillance of Thailand (NARST) among clinical isolates of *S. pneumoniae* collected from 28 hospitals in Thailand from 2000 to 2005.

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Material and Method

Isolates

The NARST, in the General Bacteriology Section, Department of Medical Sciences, Ministry of Public Health, Thailand, was established in 1998 for investigation of antimicrobial resistance situation of various pathogenic bacteria in Thailand. The program

was supported by the World Health Organization. Several activities include the standardization of microbiological laboratory practice on identification of bacteria, antimicrobial susceptibility test, and data analysis using the WHONET software program. During the 6-year period of January 1, 2000 to December 31, 2005 data collection of all routine pneumococcal isolates from the clinical microbiology laboratories of 28 coordinating study hospitals in Thailand (5 in the North, 6 in the Northeast, 5 in the Center, 4 in the East, 4 in the South, and 4 in Bangkok) was input and analyzed using the WHONET software program. All epidemiological data of each isolates were obtained at the time of enrollment in the present study.

Antimicrobial susceptibility tests

At the participating study hospitals, the identification of *S. pneumoniae* was performed using the conventional cultures and biochemical methods. *In vitro* susceptibility testing of all antimicrobials was determined by the disk diffusion method according to the recommendation of the Clinical Laboratory Standards Institute (CLSI) [formerly National Committee for Clinical Laboratory Standards (NCCLS)][8]. The minimal inhibitory concentrations (MICs) of penicillin and cefotaxime were determined by Epsilon test according to the recommendation of the manufacturer. Multi-drug-resistant (MDR) *S. pneumoniae* E-test was defined as a strain resistant to at least three antimicrobial classes. *S. pneumoniae* ATCC (American Type Culture Collection) 49169 was used as a control organism.

Statistical analysis

Epidemiological and microbiological data were obtained and analyzed by the WHONET software program. Multiple isolates from different sites of each patient were counted only one time, and antimicrobial susceptibility of the first isolate was used in the present study. A descriptive analysis was presented in terms of number and percentage.

Results

A total of 1,375, 1,310, 1,317, 1,298, 1,434, and 1,449 isolates (one isolate per one patient) of *S. pneumoniae* were collected from 2000, 2001, 2002, 2003, 2004, and 2005, respectively.

A. Antimicrobial resistance categorized by year of isolation (Tables 1-2 and Fig. 1)

Penicillin resistance: The rates of penicillin resistance of *S. pneumoniae* isolates were constantly high, ranging from 42.4% to 47.7% from 2000 to 2005. However, these rates were determined based on oxacillin-disk susceptibility test which is more reliable only with susceptible result (Table 1).

Of 6,476 pneumococcal isolates which were tested by the disk diffusion method from 2000 to 2005, only 1,103 (17%) isolates were determined for MIC by E-test. The percentages of penicillin-high-resistant, intermediate-resistant, and -susceptible strains among penicillin-non-susceptible strains tested were 89.1, 7.04, and 3.76% respectively. The percentages of penicillin-high-resistant, -intermediate-resistant, and -susceptible strains among penicillin-susceptible strains were 0.36, 2.15, and 97.5% respectively (Table 2).

Third-generation cephalosporin resistance (Table 1, Fig. 1)***:*** The rates of third-generation cephalosporin resistance were determined by E-test in only approximately 10% to 15% of all clinical isolates each year. These rates were in the range of 2.1% and 8.4%.

Table 1. Rates of antimicrobial resistance of *Streptococcus pneumoniae* isolates from 28 hospitals in Thailand from 2000 to 2005

Year	Penicillin		Cefotaxime*		Erythromycin		Clindamycin		Levofloxacin	
	NS (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.
2000	45.8	1,081	8.2	122	26.6	1,296	15.9	182	-	-
2001	45.6	1,097	4.9	204	30.3	1,273	21.8	248	0	1
2002	46.7	1,088	3.4	146	29.9	1,273	16.0	462	0	70
2003	42.8	933	8.4	119	27.8	1,180	17.5	502	0	140
2004	42.4	1,128	5.3	132	24.2	1,334	18.2	539	0	198
2005	47.7	1,149	2.1	94	28.3	1,323	17.7	555	0.3	293

NS: nonsusceptible, R: resistance, No: total number of first isolate tested per patient
* Tested by E-test

Table 2. Rates of high and intermediate penicillin resistance as well as penicillin susceptibility among penicillin-non-susceptible and penicillin-susceptible *Streptococcus pneumoniae* from 2000 to 2005

	High penicillin resistance* (%)	Intermediate penicillin resistance* (%)	Penicillin susceptibility* (%)
Penicillin nonsusceptibility** (n = 824)	89.10	7.04	3.76
Penicillinsusceptibility** (n = 279)	0.36	2.15	97.50

* Tested by E-test against penicillin, (the minimal inhibitory concentration breakpoint for penicillin high resistance ≥ 2 μg/mL, intermediate resistance = 0.12-1 mg/mL, and susceptibility ≤ 0.06 mg/mL
** Tested by the disk diffusion method against oxacillin

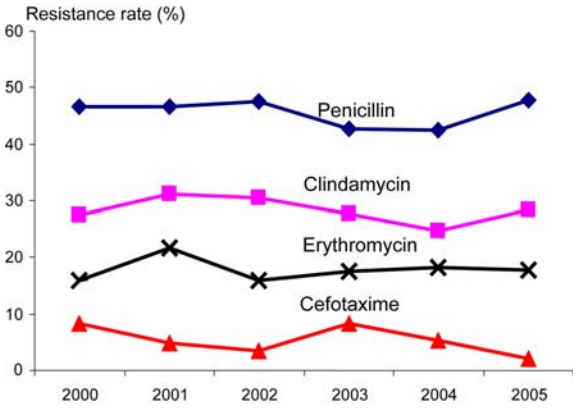


Fig. 1 Rates of antimicrobial resistance of *Streptococcus pneumoniae* isolated from 2000 to 2005 in Thailand

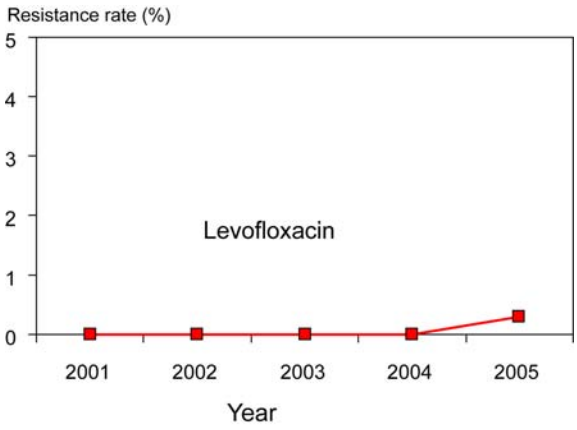


Fig. 2 Rates of levofloxacin resistance of *Streptococcus pneumoniae* isolated from 2000 to 2005 in Thailand The number in parenthesis denotes number of specimens tested

Erythromycin resistance: The rates of erythromycin resistance were relatively high, ranging from 24.2% to 30.3%.

Clindamycin resistance: The rates of clindamycin resistance were in the range of 15.9% and 21.8%.

Fluoroquinolone resistance: Only a small number of clinical isolates each year were determined for levofloxacin susceptibility. Surprisingly, one isolate in 2005 was resistant to levofloxacin.

Multi-drug resistance: The rates of multi-drug resistance ranged from 14.8% to 34.3%.

B. Antimicrobial resistance categorized by the geographic distribution (Tables 3-7 and Fig. 3)

In the present study, Thailand was arbitrarily divided into five regions including the North, the Northeast, the Center, the East, and the South as well as Bangkok (the capital).

Penicillin resistance (Table 3)***:*** The rates of nonsusceptible penicillin determined by oxacillin-disk test varied widely from 31.8% to 73.1% among isolates from each region, except those from the Northeast in 2000 which had a penicillin resistance rate of 18.9%. The high rates of penicillin resistance in the range of 40% and 60% were observed among isolates from the North, the Center, the East, and Bangkok, compared with the low rates between 30% and 40% in those from the Northeast and the South. The rates of penicillin resistance have markedly increased from 18.9% in 2000 to 40.2% in 2005 among isolates from the Northeast.

Third-generation cephalosporin resistance (Table 4)***:*** The rates of third-generation cephalosporin resistance were not significantly different among isolates from each region and Bangkok. However, these rates among isolates from the Northeast varied widely from 3.8% in 2002 to 22.2% in 2003.

Erythromycin resistance (Table 5)***:*** The rates of erythromycin resistance ranged from 22.4% to 39.1% among isolates from the North, the Center, and the East. The highest rates (39.5% to 50%) were

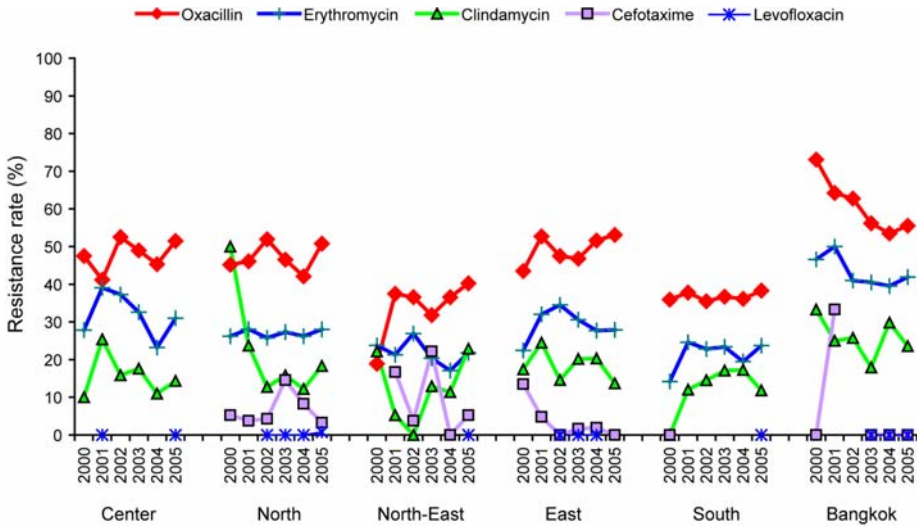


Fig. 3 Rates of antimicrobial resistance of *Streptococcus pneumoniae* isolated from different parts of Thailand

Table 3. Rates of penicillin-nonsusceptible *Streptococcus pneumoniae* isolates from 2000 to 2005 by region

Year	North		Northeast		East		Center		Bangkok		South	
	NS (%)	No.	NS (%)	No.	NS (%)	No.	NS (%)	No.	NS (%)	No.	NS (%)	No.
2000	45.2	445	18.9	282	43.5	186	47.5	40	73.1	26	35.9	103
2001	46.1	397	37.5	208	52.7	150	41.2	80	64.2	106	37.8	156
2002	51.9	345	36.6	290	47.5	101	52.5	40	62.7	161	35.5	152
2003	46.5	288	31.8	195	46.8	126	49.0	51	56.2	96	36.7	177
2004	42.1	278	36.6	328	51.6	153	45.3	86	53.5	114	36.1	169
2005	50.8	297	40.2	259	53.1	177	51.5	134	55.5	128	38.3	154

NS: nonsusceptible, R: resistance, No.: total number of isolates tested

Table 4. Rates of cefotaxime-resistant* *Streptococcus pneumoniae* isolates from 2000 to 2005 by the region

Year	North		Northeast		East		Center		Bangkok		South	
	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.
2000	5.3	57	-	-	13.5	52	-	-	0	11	0	2
2001	3.8	130	16.7	6	4.8	62	-	-	33.3	3	-	-
2002	4.3	93	3.8	26	0	27	-	-	-	-	-	-
2003	14.6	48	22.2	9	1.7	60	-	-	0	2	-	-
2004	8.3	72	0	5	1.9	54	-	-	0	2	-	-
2005	3.3	30	5.3	19	0	42	-	-	0	2	-	-

R: resistance, No.: total number of isolates tested
* Tested by E-test

observed among isolates from Bangkok, compared to the lower rate among isolates from the Northeast (17.1% to 26.9%) and the South (14.2 to 24.6%).

Clindamycin resistance (Table 6)***:*** The rates of clindamycin resistance ranged from 5.3% to 25.4%, and were not different among isolates from each region except those from Bangkok with relatively higher rates of resistance between 17.9% and 33.3%.

Fluoroquinolone resistance (Table 7)***:*** A definite comparison regarding fluoroquinolones resistance among isolates from each region and Bangkok could not be made due to the too small sample

Table 5. Rates of erythromycin-resistant *Streptococcus pneumoniae* isolates from 2000 to 2005 by region

Year	North		Northeast		East		Center		Bangkok		South	
	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.
2000	26.2	454	23.8	298	22.4	196	27.8	97	46.6	146	14.2	106
2001	28.1	409	21.3	239	32.0	178	39.1	161	50.0	118	24.6	171
2002	25.8	361	26.9	309	34.5	139	37.3	134	41.0	173	22.8	158
2003	27.3	293	20.4	245	30.6	147	33.2	187	40.5	116	23.4	192
2004	26.2	275	17.1	339	27.7	166	23.2	207	39.5	162	19.5	185
2005	28.0	300	21.8	284	27.9	183	31.0	216	41.9	167	23.7	173

R: resistance, No.: total number of isolates tested

Table 6. Rates of clindamycin-resistant *Streptococcus pneumoniae* isolated from 2000 to 2005 by region

Year	North		Northeast		East		Center		Bangkok		South	
	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.
2000	50.0	2	22.2	9	17.4	86	10.0	70	33.3	12	0	3
2001	23.7	38	5.3	19	24.5	94	25.4	59	25.0	12	12.0	25
2002	12.7	150	0	14	14.6	82	15.9	63	25.8	93	14.5	62
2003	15.8	101	12.9	31	20.2	114	17.6	85	17.9	95	17.1	76
2004	12.2	115	11.4	44	20.3	118	11.0	73	29.8	114	17.3	75
2005	18.3	131	22.9	48	13.7	146	14.3	56	23.6	123	11.8	51

R: resistance, No.: total number of isolates tested

Table 7. Rates of levofloxacin-resistant *Streptococcus pneumoniae* isolates from 2000 to 2005 by region

Year	North		Northeast		East		Center		Bangkok		South	
	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.
2000	-	-	-	-	-	-	-	-	-	-	-	-
2001	-	-	-	-	-	-	0	1	-	-	-	-
2002	0	66	-	-	0	5	-	-	-	-	-	-
2003	0	131	-	-	0	6	-	-	0	3	-	-
2004	0	170	-	-	0	19	-	-	0	10	-	-
2005	0	120	0	52	-	-	0	1	0	102	0	18

R: resistance, No.: total number of isolates tested

size. One of 120 strains (0.083%) with levofloxacin resistance was isolated from the North in the year 2005.

C. Antimicrobial categorized by the hospital type (Tables 8-12 and Fig. 4)

In the present study, there were four types of hospitals including provincial hospital (between 100 and 400 beds), regional hospital (between 400 and 1,000 beds), university hospital, and private hospital. Most of the isolates (approximately 700-900 isolates each year) were from the regional hospitals, compared to only 10-100 isolates each year from other types of hospitals.

Penicillin resistance (Table 8)***:*** The highest rates of penicillin resistance (63.9% to 85.7%) were observed among isolates from university hospitals, compared to those between 26.5% and 48.6% from provincial and regional hospitals, and those between 22.7% and 68.4% from private hospitals.

Third-generation cephalosporins resistance (Table 9)***:*** A definite comparison regarding third-generation cephalosporin resistance among isolates from each type of hospitals could not be made due to the too small sample size.

Erythromycin resistance (Table 10)***:*** The high rates of erythromycin resistance were observed among isolates from university (42.6% to 50%) and private (32.6% to 50%) hospitals, compared to those between 16.4% and 38.3% from provincial and regional hospitals. There was an increasing rates of erythromycin resistance from 16.4% in 2000 to 38.3% in 2005 among isolates from provincial hospitals.

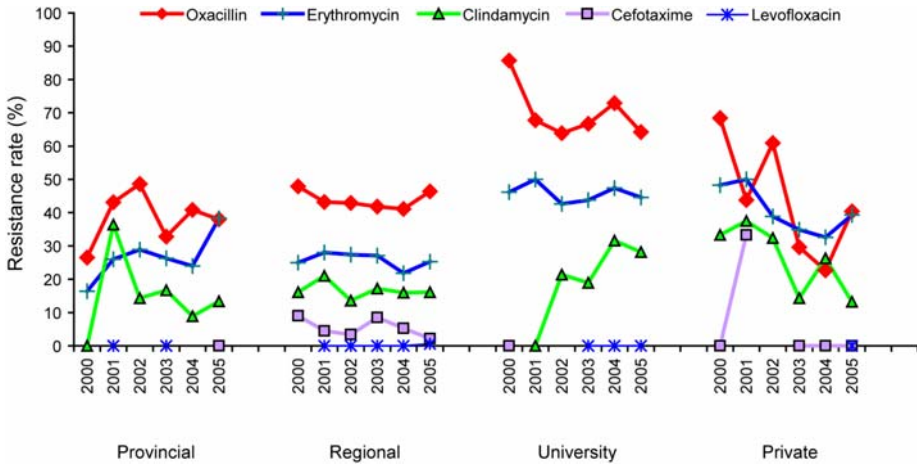


Fig. 4 Rates of antimicrobial resistance of *Streptococcus pneumoniae* isolated from different types of hospitals in Thailand

Table 8. Rates of penicillin-nonsusceptible *Streptococcus pneumoniae* isolates from 2000 to 2005 by type of hospital

Year	Provincial hospitals		Regional hospitals		University hospitals		Private hospitals	
	NS (%)	No.	NS (%)	No.	NS (%)	No.	NS (%)	No.
2000	26.5	68	47.9	937	85.7	7	68.4	19
2001	43.1	123	43.2	864	67.8	90	43.8	16
2002	48.6	70	42.9	843	63.9	97	60.9	64
2003	32.8	64	41.8	720	66.7	69	29.6	27
2004	40.8	76	41.1	886	72.9	70	22.7	44
2005	38.0	79	46.4	870	64.2	81	40.4	47

R: resistance, No.: total number of isolates tested

Clindamycin resistance (Table 11)***:*** The high rates of clindamycin resistance were observed among isolates from university (18.9% to 31.6%) and private (13.2% to 37.5%) hospitals, compared to those between 8.9% and 36.4% from provincial and regional hospitals. There was an increasing trend of clindamycin resistance among isolates from university hospitals, in contrast to a wide variation of resistance rates among those from private hospitals.

Fluoroquinolone resistance (Table 12)***:*** There was only one isolate with levofloxacin resistance from the regional hospital.

Table 9. Rates of cefotaxime-resistant* *Streptococcus pneumoniae* isolates from 2000 to 2005 by type of hospital

Year	Provincial hospitals		Regional hospitals		University hospitals		Private hospitals	
	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.
2000	-	-	9.0	111	0	10	0	1
2001	-	-	4.5	199	-	-	33.3	3
2002	-	-	3.4	146	-	-	-	-
2003	-	-	8.5	117	-	-	0	2
2004	-	-	5.3	131	-	-	0	2
2005	0	1	2.2	91	-	-	0	2

R: resistance, No.: total number of isolates tested
* Tested by E-test

Table 10. Rates of erythromycin-resistant *Streptococcus pneumoniae* isolates from 2000 to 2005 by type of hospital

Year	Provincial hospitals		Regional hospitals		University hospitals		Private hospitals	
	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.
2000	16.4	73	25.0	1,027	46.2	117	48.3	29
2001	26.0	131	28.0	971	50.0	100	50.0	18
2002	28.9	76	27.4	959	42.6	101	38.9	72
2003	26.3	133	27.1	873	43.8	73	34.9	43
2004	24.0	121	21.8	993	47.4	76	32.6	86
2005	38.3	115	25.3	967	44.6	83	39.3	84

R: resistance, No.: total number of isolates tested

Table 11. Rates of clindamycin-resistant *Streptococcus pneumoniae* isolates from 2000 to 2005 by type of hospital

Year	Provincial hospitals		Regional hospitals		University hospitals		Private hospitals	
	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.
2000	0	14	16.1	155	-	-	33.3	12
2001	36.4	11	21.0	219	0	4	37.5	8
2002	14.3	14	13.6	353	21.4	56	32.4	37
2003	16.7	24	17.2	379	18.9	74	14.3	21
2004	8.9	56	16.0	368	31.6	76	26.3	38
2005	13.4	67	16.1	354	28.2	85	13.2	38

R: resistance, No.: total number of isolates tested

Table 12. Rates of levofloxacin-resistant *Streptococcus pneumoniae* isolated from 2000 to 2005 by type of hospital

Year	Provincial hospitals		Regional hospitals		University hospitals		Private hospitals	
	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.
2000	-	-	-	-	-	-	-	-
2001	0	1	0	1	-	-	-	-
2002	-	-	0	70	-	-	-	-
2003	0	2	0	135	0	3	-	-
2004	-	-	0	189	0	10	-	-
2005	-	-	0.6	173	0	67	0	35

R: resistance, No.: total number of isolates tested

D. Antimicrobial resistance categorized by the age group (Tables 13-17 and Fig. 5)

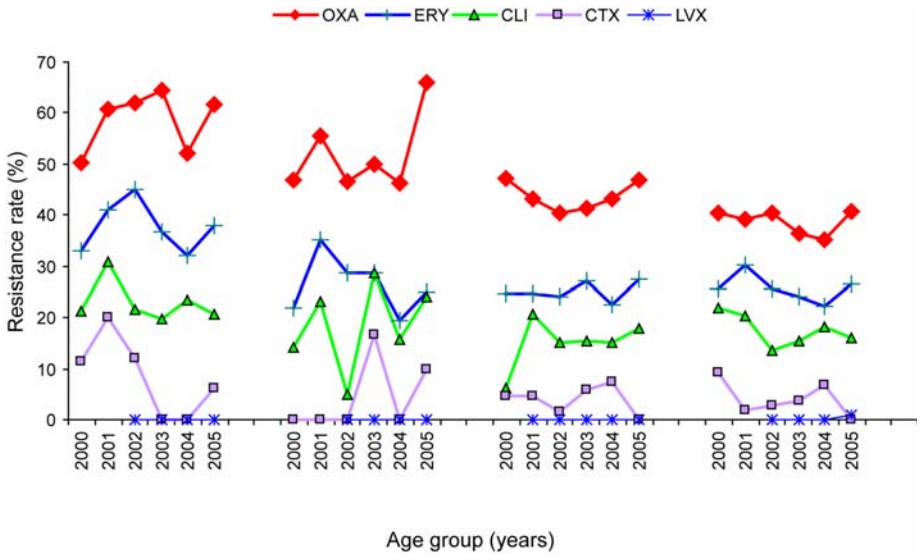
In the present study, there were four age groups including the age group of less than six years (young child), between 6-15 years, (old child) between 16-60 years (adults), and of more than 60 years (elderly). Most of the data were from the groups of young child, adults, and elderly.

Penicillin resistance (Table 13)***:*** The highest penicillin resistance rates were observed among isolates from the young (50.3% to 64.6%) and old (46.2% to 66%) children groups, compared to those between 40.5% and 47.2% from the adult group, and between 35.3% and 40.8% from the elderly group.

Third-generation cephalosporin resistance (Table 14)***:*** A comparison among isolates from each age group could not be made due to the small sample size.

Erythromycin resistance (Table 15)***:*** The rates of erythromycin resistance were higher among isolates from the young child group (32% to 45.1%) than those of old child (19.4% to 35.1%), adult (22.6% to 27.5%), and elderly (22.1% to 30.2%) groups.

Clindamycin resistance (Table 16)***:*** Similarly, the rates of clindamycin resistance were higher



OXA : oxacillin, ERY : erythromycin, CLI : clindamycin, CTX : cefotaxime, LVX : levofloxacin

Fig. 5 Rates of antimicrobial resistance of *Streptococcus pneumoniae* isolated from patients of different age groups in Thailand

Table 13. Rates of penicillin-nonsusceptible *Streptococcus pneumoniae* isolates from 2000 to 2005 by age group

Year	< 6 years old		6-15 years old		15-60 years old		> 60 years old	
	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.
2000	50.3	167	46.9	49	47.2	360	40.4	324
2001	60.7	214	55.6	72	43.1	413	39.2	352
2002	62.1	243	46.5	71	40.5	393	40.3	325
2003	64.6	130	50.0	40	41.4	314	36.3	342
2004	52.0	175	46.2	52	43.2	417	35.3	402
2005	61.8	204	66.0	50	46.9	441	40.8	434

R: resistance, No.: total number of isolates tested

Table 14. Rates of cefotaxime-resistant* *Streptococcus pneumoniae* isolated from 2000 to 2005 by age group

Year	< 6 years old		6-15 years old		15-60 years old		> 60 years old	
	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.
2000	11.5	26	0	8	4.5	44	9.1	33
2001	20.0	25	0	20	4.5	89	1.7	58
2002	12.1	33	0	11	1.6	64	2.9	35
2003	0	17	16.7	6	6.0	50	3.7	27
2004	0	6	0	10	7.5	53	6.7	45
2005	6.2	16	10.0	10	0	37	0	25

R: resistance, No.: total number of isolates tested
* Tested by E-test

Table 15. Rates of erythromycin-resistant *Streptococcus pneumoniae* isolated from 2000 to 2005 by age group

Year	< 6 years old		6-15 years old		15-60 years old		> 60 years old	
	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.
2000	33.1	260	21.8	55	24.6	390	25.7	374
2001	41.0	239	35.1	77	24.8	476	30.2	407
2002	45.1	275	28.8	80	24.1	460	25.7	385
2003	36.8	171	28.6	49	27.0	415	24.0	420
2004	32.0	206	19.4	62	22.6	495	22.1	494
2005	37.9	235	25.0	64	27.5	513	26.5	494

R: resistance, No.: total number of isolates tested

among isolates from the young child group (19.8% to 30.8%) than those of other age groups (5% to 28.6%). During the study period, the rates were constant among isolates from each age group.

Fluoroquinolone resistance (Table 17)***:*** A comparison could not be made because only one isolate with levofloxacin resistance from the elderly group was identified.

E. Antimicrobial resistance categorized by the type of clinical specimen (Tables 18-22)

In the present study, there were two main

Table 16. Rates of clindamycin-resistant *Streptococcus pneumoniae* isolates from 2000 to 2005 by age group

Year	< 6 years old		6-15 years old		15-60 years old		> 60 years old	
	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.
2000	21.4	28	14.3	7	6.2	64	21.8	55
2001	30.8	26	23.1	13	20.8	96	20.2	99
2002	21.6	102	5.0	20	15.2	178	13.5	148
2003	19.8	96	28.6	21	15.5	187	15.4	162
2004	23.3	116	15.8	19	15.0	193	18.1	204
2005	20.8	120	24.1	29	18.0	211	16.1	193

R: resistance, No.: total number of isolates tested

Table 17. Rates of levofloxacin-resistant of *Streptococcus pneumoniae* isolates from 2000 to 2005 by age group

Year	< 6 years old		6-15 years old		15-60 years old		> 60 years old	
	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.
2000	-	-	-	-	-	-	-	-
2001	-	-	-	-	0	1	-	-
2002	0	5	0	5	0	29	0	34
2003	0	8	0	12	0	47	0	44
2004	0	18	0	10	0	74	0	79
2005	0	82	0	13	0	99	1	97

R: resistance, No.: total number of isolates tested

types of clinical specimens including those from sterile and non-sterile body sites. The specimens were obtained from cerebrospinal fluid (CSF), blood, respiratory, and other sterile body sites as detailed in Table 18. The specimens from non-sterile sites were respiratory and non-respiratory specimens.

Penicillin resistance (Table 18)***:*** The rates of penicillin resistance were higher among isolates from respiratory (44.2% to 51.3%) and non-respiratory non-sterile (35.7% to 55.1%) sites, than those from CSF (32.4% to 47.8%), blood (29.5% to 39.2%), respiratory sterile (9.1% to 33.3%), and other sterile (20% to 33.3%) sites.

Third-generation cephalosporin resistance (Table 19)***:*** A comparison could not be made due to the small sample size.

Erythromycin resistance (Table 20)***:*** The rates of erythromycin resistance were higher among isolates from non-sterile specimens (11.1% to 36.2%) than those from sterile specimens (9.1% to 30%). During the study period, the rates were constant among isolates from each type of specimens.

Clindamycin resistance (Table 21)***:*** The rates of clindamycin resistance were higher among isolates from non-sterile sites (7.1% to 30.4%) than those from sterile sites (8.8% to 40%). However, a definite comparison could not be made due to the small sample size from each type of specimens.

Fluoroquinolone resistance (Table 22)***:*** A comparison could not be made because to only one isolate with levofloxacin resistance from a respiratory non-sterile specimen was identified.

Discussion

The present study indicates that the rates of penicillin non-susceptibility in Thailand were constantly high, ranging from 42.4% in 2000 to 47.7% in 2005, especially among isolates from the North, the Center, the East, and Bangkok; from university hospitals; from young children; and from non-sterile specimens. Most of these isolates were intermediately resistant to penicillin. In contrast to penicillin resistance, the rates of

Table 18. Rates of penicillin-nonsusceptible *Streptococcus pneumoniae* isolates from 2000 to 2005 by type of specimen

Year	Cerebrospinal fluid and brain tissue		Blood		Respiratory sterile site[1]		Other sterile site[2]		Respiratory non-sterile site[3]		Non-respiratory non-sterile site[4]	
	NS (%)	No.	NS (%)	No.	NS (%)	No.	NS (%)	No.	NS (%)	No.	NS (%)	No.
2000	34.2	38	31.7	189	33.3	6	33.3	6	49.3	767	46.8	94
2001	32.4	34	32.6	227	27.3	11	0	4	49.9	746	48.1	79
2002	32.6	46	33.5	215	25.0	12	22.2	9	50.1	706	49.5	93
2003	36.1	36	38.1	160	33.3	6	28.6	7	44.2	640	43.3	60
2004	47.8	46	29.5	251	9.1	11	20.0	10	48.4	715	35.7	70
2005	32.4	37	39.2	319	28.6	7	0	3	51.3	679	55.1	49

NS: nonsusceptible, No.: total number of isolates tested
[1] Respiratory sterile site : pleural fluid, lung necropsy
[2] Other sterile site : joint fluid, abdominal fluid, fluid
[3] Respiratory non-sterile site : ear, nose, sinus, throat swab, upper respiratory, tracheal aspirate, sputum, bronchus, bronchoalveolar lavage
[4] Bile, urine, pus, cervix, drainage, rectal swab, genitalia

Table 19. Rates of cefotaxime resistance* of *Streptococcus pneumoniae* isolates from 2000 to 2005 by type of specimen

Year	Cerebrospinal fluid and brain tissue		Blood		Respiratory sterile site[1]		Other sterile site[2]		Respiratory non-sterile site[3]		Non-respiratory non-sterile site[4]	
	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.
2000	0	3	3	33	0	3	0	1	10.3	78	12.5	8
2001	0	6	3.1	65	0	2	-	-	5.9	118	7.1	14
2002	0	7	4.9	61	0	3	0	2	2.7	74	0	9
2003	11.1	9	0	49	0	3	0	1	15.3	59	0	5
2004	0	10	0	43	0	2	0	1	7.7	78	25.0	4
2005	0	7	0	35	0	3	-	-	1.9	52	33.3	3

R: resistance, No.: total number of isolates tested
* Tested by E-test
[1] Pleural fluid, lung necropsy, autopsy.
[2] Joint fluid, abdominal fluid, fluid.
[3] Eye,ear, nose, sinus, throat swab, upper respiratory tract, tracheal aspirate, sputum, bronchus, bronchoalveolar lavage
[4] Bile, urine, pus, cervix, drainage, rectal swab, genitalia

cefotaxime resistance were very low, ranging from 2.1% to 8.4%.

The high and increasing prevalence of penicillin resistance in *S. pneumoniae* was consistent with several reports in the United States[5,9-11], Canada[12], Europe[13,14], Africa[2], and Asia[7]. An international surveillance study conducted from 2000 to 2001 in 12 Asian countries including Thailand by the Asian Network for Surveillance of Resistant Pathogens (ANSORP) documented the rates of penicillin resistance among clinical *S. pneumoniae* isolates in the range between 7.8% (India) and 92% (Vietnam), with the mean resistance rate of 52.4% (intermediate and high resistance of 23% and 29.4%)[7]. Among 52 isolates from Chulalongkorn University, Bangkok, Thailand in the ANSORP study, there were 26.9% and 26.9% of penicillin intermediate and high resistance, respectively. This observation is different

Table 20. Rates of erythromycin-resistant *Streptococcus pneumoniae* isolated from 2000 to 2005 by type of specimen

Year	Cerebrospinal fluid and brain tissue		Blood		Respiratory sterile site[1]		Other sterile site[2]		Respiratory non-sterile site[3]		Non-respiratory non-sterile site[4]	
	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.
2000	24.1	54	17.2	215	9.1	11	16.7	6	29.4	931	23.1	104
2001	30	40	21.5	251	25	12	0	5	32.4	854	29.3	99
2002	12.7	55	20.3	236	0	10	0	10	32.9	828	34.3	102
2003	20.5	44	27.5	204	12.5	8	9.1	11	28	783	36.2	69
2004	29.6	54	20.7	294	9.1	11	0	9	27	841	11.1	81
2005	22	41	19.1	341	0	8	0	3	32.3	805	22.2	54

R: resistance, No.: total number of isolates tested
[1] Pleural fluid, lung necropsy, autopsy
[2] Joint fluid, abdominal fluid, fluid
[3] Eye,ear, nose, sinus, throat swab, upper respiratory aspirate, tracheal, sputum, bronchus, bronchoalveolar lavage
[4] Bile, urine, pus, cervix, drainage, rectal swab, genitalia

Table 21. Rates of clindamycin-resistant *Streptococcus pneumoniae* isolated from 2000 to 2005 by type of specimen

Year	Cerebrospinal fluid and brain tissue		Blood		Respiratory sterile site[1]		Other sterile site[2]		Respiratory non-sterile site[3]		Non-respiratory non-sterile site[4]	
	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.
2000	0	12	15.8	38	0	1	-	-	17.5	120	20	10
2001	40	5	21.4	56	20	5	0	1	22.3	166	0	8
2002	11.1	18	9.2	98	0	3	0	7	17.7	288	18.2	33
2003	12.5	16	17	100	0	6	0	4	18.2	335	30.4	23
2004	36.4	22	17.1	117	0	4	0	5	19.2	365	7.1	14
2005	15.4	26	8.8	148	0	5	0	2	20.4	324	27.8	18

R: resistance, No.: total number of isolates tested
[1] Pleural fluid, lung necropsy, autopsy
[2] Joint fluid, abdominal fluid, fluid
[3] Eye,ear, nose, sinus, throat swab, upper respiratory aspirate, tracheal, sputum, bronchus, bronchoalveolar lavage
[4] Bile, urine, pus, cervix, drainage, rectal swab, genitalia

from the results of the present study which revealed high-level penicillin-resistant *S. pneumoniae* in 89.1% of penicillin-nonsusceptible isolates.

In the present study, the rates of erythromycin resistance were relatively high, ranging from 24.2% to 30.3%, consistent with previous studies in the United States[5,9-11], Canada[12], Europe[13,14], and Asia[7]. The ANSORP study described the erythromycin resistance rate of 54.9% among isolates from 12 Asian countries. Among 52 isolates from Thailand, there were 5.8% and 36.5% of erythromycin intermediate and high resistance, respectively, consistent with the results of the present study. In the present study, the rates of erythromycin resistance were higher among isolates from university and private hospitals as well as from young children. These observations were similar to those described by the ANSORP study[7].

One strain of *S. pneumoniae* with levofloxacin resistance was isolated in a patient older than 60 years in 2005. In contrast, no isolate with levofloxacin resistance from Thailand was noted in the ANSORP

Table 22. Rates of levofloxacin resistance of *Streptococcus pneumoniae* isolated from 2000 to 2005 type of specimen

Year	Cerebrospinal fluid and brain tissue		Blood		Respiratory sterile site[1]		Other sterile site[2]		Respiratory non-sterile site[3]		Non-respiratory non-sterile site[4]	
	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.
2000	-	-	-	-	-	-	-	-	-	-	-	-
2001	-	-	0	1	-	-	-	-	-	-	-	-
2002	0	1	0	12	-	-	0	3	0	40	0	8
2003	0	4	0	28	0	1	-	-	0	86	0	14
2004	0	6	0	38	0	1	0	3	0	137	0	9
2005	0	8	0	73	-	-	-	-	0.5	198	0	10

R: resistance, No.: total number of isolates tested
[1] Pleural fluid, lung necropsy, autopsy
[2] Joint fluid, abdominal fluid, fluid
[3] Eye,ear, nose, sinus, throat swab, upper respiratory aspirate, tracheal, sputum, bronchus, bronchoalveolar lavage
[4] Bile, urine, pus, cervix, drainage, rectal swab, genitalia

study[7]. However, 3.8% of those isolates were resistant to ciprofloxacin.

In accordance with the ANSORP study we noticed that the rates of MDR *S. pneumoniae* ranged from 14.8% to 34.3%.

Based on the above results of the present study, third-generation cephalosporin should be the antimicrobial of choice for empirical treatment of infections caused by *S. pneumoniae*, especially meningitis, before obtaining the microbiology results in Thailand. However, the rates of third-generation cephalosporin resistance could not be exactly determined due to too small sample size (only 10% to 15% of all isolates each year) in the present study. Thus, a recommendation regarding the combination of third-generation cephalosporin and vancomycin or rifampin for empirical treatment of pneumococcal meningitis cannot be made unless the data of cephalosporin susceptibility results are available in all isolates. In addition, the antimicrobial agent for the empirical treatment of community-acquired pneumonia (CAP) in Thailand should not be erythromycin alone especially in pediatric patients. Even though most *S. pneumoniae* isolates remained susceptible to levofloxacin, the results of the present study suggest that the incidence of resistance to this antimicrobial is slowly increasing. These data raise another warning signal with regard to the emergence and spread of fluoroquinolone resistance, and may affect the guidelines of empirical treatment of CAP with levofloxacin or other respiratory quinolones in Thailand. Clinical failures of levofloxacin in the treatment of CAP have been documented in patients infected with *S. pneumoniae* with reduced susceptibility to levofloxacin[15].

Our study has some limitations in interpreting the data. Since *S. pneumoniae* isolates were obtained from only 28 hospitals in Thailand and the number of isolates was relatively low in some types of hospitals and age groups, the results of the present study may not reflect the overall resistance rates in the whole country. Therefore, further surveillance with more isolates from more hospitals and some age groups is strongly warranted. In addition, the susceptibility testing of some antimicrobials including penicillin (both intermediate and high resistance), third-generation cephalosporins, macrolides other than erythromycin, and fluoroquinolones both levofloxacin and other respiratory quinolones should be performed.

Moreover, in the present study, the direct relationship between known risk factors and anti-microbial resistance in *S. pneumoniae* cannot be determined. The present study did not demonstrate the serotype distributions among clinical isolates especially the serotypes contained in the pneumococcal vaccine. Given the high antimicrobial resistance rates of *S. pneumoniae*, vaccination should be applied more widely in Thailand. Thus, the data of the serotypes of *S. pneumoniae* circulating in Thailand would be beneficial to determine the cost-effectiveness of pneumococcal vaccination.

In conclusion, the present (NARST) study documents remarkable increases in the penicillin, erythromycin, and multi-drug resistance rates in Thailand. The injudicious use of antibiotics and the clonal spread of these resistant strains may be the major reasons for these high resistance rates. Continuous surveillance among *S. pneumoniae* isolates as well as appropriate use of antimicrobial and pneumococcal vaccination are seriously needed in Thailand.

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การเฝ้าระวังการดื้อยาของเชื้อ Streptococcus pneumoniae ในประเทศไทย

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จากส่วนหนึ่งของการเฝ้าระวังเชื้อดื้อยาแห่งชาติอย่างต่อเนื่องโดย National Antimicrobial Resistance Surveillance Thailand (NARST) พบว่า Streptococcus pneumoniae ที่แยกได้จากสิ่งส่งตรวจทางคลินิกของโรงพยาบาลทั่วประเทศไทยระหว่างปี พ.ศ. 2543-2548 โดยลงข้อมูลทางจุลชีววิทยาและระบาดวิทยาที่ได้จากแต่ละโรงพยาบาล และวิเคราะห์โดยใช้โปรแกรม WHONET ผลการทดสอบความไวต่อยาต้านจุลชีพด้วยวิธีมาตรฐาน oxacillin disk diffusion จากเชื้อในผู้ป่วยทั้งหมด 6,476 คน พบอัตราการดื้อ penicillin ในระหว่าง 6 ปี อยู่ในอัตราสูงระหว่างร้อยละ 42.4-47.7 และมีการนำเชื้อที่ดื้อ penicillin จากผู้ป่วย 824 คน ทดสอบด้วยวิธี Epsilon พบว่าดื้อในระดับสูง ร้อยละ 89.1 ดื้อในระดับปานกลาง ร้อยละ 7.04 และ ไวต่อ penicillin ร้อยละ 3.76 สำหรับ cephalosporin ในรุ่นที่ 3 พบว่ามีการทดสอบด้วยวิธี Epsilon เพียงร้อยละ 10-15 ของจำนวนเชื้อทั้งหมด โดยมีอัตราการดื้อนี้อยู่ระหว่างร้อยละ 2.1-8.4 จากการทดสอบวิธี disk diffusion พบอัตราดื้อ erythromycin อยู่ระหว่างร้อยละ 24.6-31.1 และที่น่าสนใจคือพบ 1 เชื้อที่แยกได้ในปี พ.ศ. 2548 ดื้อยา levofloxacin สำหรับอัตราการดื้อยาต้านจุลชีพตั้งแต่ 3 กลุ่มขึ้นไป อยู่ระหว่างร้อยละ 14.8-34.3

ผลสรุปจากการศึกษานี้พบเชื้อ S. pneumoniae ในประเทศไทยมีอัตราการดื้อยาเพ็นนิซิลลิน erythromycin และดื้อยาต้านจุลชีพตั้งแต่ 3 กลุ่มขึ้นไป มีอัตราสูง โดยเฉพาะเชื้อที่แยกได้จากภาคเหนือ ภาคกลาง ภาคตะวันออก และกรุงเทพมหานคร จากโรงพยาบาลมหาวิทยาลัย จากผู้ป่วยเด็ก และจากสิ่งส่งตรวจที่ไม่ปลอดเชื้อ (non-sterile sites)