CHAPTER 4

RESULT

4.1 Part 1: Assessment of Prevalence of Drug-Resistant *Streptococcus pneumoniae* in Southern Thailand

4.1.1 Overall Susceptibility

One hundred and twenty seven of 286 *Streptococcus pneumoniae* isolates from 15 hospitals were eligible for susceptibility test. Fifty-nine isolates died during transportation. Only 92 patient medical records (72%) were available for reviewing. Therefore, 92 pneumococcal isolates from patients with pnemococcal infection were included in this study. The most common specimen from which *S. pneumoniae* were isolated was sputum (60%) followed by blood (29%) and CSF (11%). Susceptibility of pneumococci to penicillin in each province was shown in Table 10. Songkhla Province was the area, where, we obtained the most pneumococcal isolates, and could determine prevalence of penicillin-susceptible, -intermediate susceptible and -resistant pneumococci as followed: 50%, 42.9% and 7.1%, respectively. The percentage of penicillin susceptibility in other regions could not be determined due to small number of isolate received. However, if we separated the region into 2 areas: upper and lower Southern area, prevalence of penicillin-susceptible, -intermediate susceptible and -resistant pneumococci in upper Southern area was as followed: 31%, 62% and 6.9%, respectively. The percentage of penicillin-susceptible, -intermediately susceptible and -resistant isolates in lower Southern area was 49.2%, 42.8% and 7.9%, respectively. Overall, 43.5% isolates were susceptible to penicillin, 48.9% showed intermediate susceptibility and only 7.6% of the isolates were resistant to penicillin. Thus, prevalence

of penicillin-nonsusceptible *Streptococcus pneumoniae* isolates from patients with community-acquired infection in Southern Thailand was 56.5%. Table 11 illustrated the susceptibility prevalence of pneumococci to selected antibiotic agents.

Table 10 Susceptibility of *Streptococcus pneumoniae* to penicillin in Southern Thailand stratified by province.

Province	S (*n*=40)	I (*n*=45)	R (*n*=7)	I+R (%)	Total
Songkhla	21 (50%)	18 (42.9%)	3 (7.1%)	21 (50%)	42 (45.7%)
Nakhonsithammarat	3 (25%)	9 (75%)	0	9 (75%)	12 (13%)
Suratthani	3 (37.5%)	4 (50%)	1 (12.5%)	5(62.5%)	8 (8.7%)
Trang	1 (16.7%)	3 (50%)	2 (33.3%)	5(83.3%)	6 (6.5%)
Narathiwat	5 (100%)	0	0	0	5 (5.4%)
Yala	3 (60%)	2 (40%)	0	2(40%)	5 (5.4%)
Chumporn	1 (33.3%)	2 (66.7%)	0	2(66.7%)	3 (3.3%)
Puket	2 (66.7%)	0	1 (33.3%)	1(33.3%)	3 (3.3%)
Patthalung	0	2 (100%)	0	2(100%)	2 (2.2%)
Pattani	1 (50%)	1 (50%)	0	1(50%)	2 (2.2%)
Pung-nga	0	2 (100%)	0	2(100%)	2 (2.2%)
Krabi	0	1 (100%)	0	1(100%)	1 (1.1%)
Satul	0	1 (100%)	0	1(100%)	1 (1.1%)
Total	(43.5%)	(48.9%)	(7.6%)	(56.5%)	(*n*=92)

S = susceptible, I = intermediate susceptibility, R = resistant

Table 11 Prevalence of drug resistant *Streptococcus pneumoniae* in Southern Thailand stratified by selected antibiotic agents.

Antibiotic agents	S (%)	I (%)	R (%)	I+R (%)
Penicillin, *n*=92	43.5	48.9	7.6	56.5
Cefotaxime (meningitis), *n*=10	100	0	0	0
Cefotaxime (non-meningitis), *n*=82	95.1	4.9	0	4.9
Imipenem, *n*=92	71.7	28.3	0	28.3
Levofloxacin, *n*=92	100	0	0	0
Erythromycin, *n*=92	56.5	1.1	42.4	43.5

S = susceptible, I = intermediate susceptibility, R = resistant.

NCCLS breakpoint (μg/ml): penicillin S=≤0.06, I=0.12-1, R=≥2;

cefotaxome (meningitis) S=≤0.5, I=1, R=≥2;

cefotaxime (non-meningitis) S=≤1, I=2, R=≥4; imipenem S=≤0.12, I=0.25-0.5, R=≥1;

levofloxacin S=≤2, I=4, R=≥8; erythromycin S=≤0.25, I=0.5, R=≥1

Multidrug-resistant pneumococci were determined as isolate that resist to three or more of different antibiotic classes. (Crook and Spratt, 1998; Chenoweth, *et al*, 2000; Harwell and Brown, 2000). Prevalence of multidrug-resistant pneumococci in this study was 22.8%. Three clinical isolates from sputum of patients with pneumonia were resistant to 4 antibiotic agents, and 18 clinical isolates were resistant to 3 antibiotic agents (Table 12).

Table 12 Multidrug-resistant pneumococci in Southern Thailand.

Penicillin	Cefotaxime	Imipenem	Levofloxacin	Erythromycin	No.(%)
R	R	R	S	R	3 (3.2%)
R	R	R	S	S	1 (1.1%)
R	S	R	S	R	17 (18.5%)

S = susceptible, R = nonsusceptible (intermediate susceptible + resistant)

4.1.2 Susceptibilities stratified by Site of Infection

According to newest NCCLS guideline published in 2002, cefotaxime MIC breakpoint of *Streptococcus pneumoniae* was classified into 2 groups: meningitis and non-meningitis. Hence, susceptibility of pneumococci to cefotaxime isolated from patients with meningitis and those from patients with nonmeningeal infection was determined separately according to the source of bacteria isolated. All of pneumococcal isolates from CSF were susceptible to cefotaxime. When the isolates from blood (n=27) and CSF (n=10) were combined and used NCCLS meningitis cefotaxime breakpoint to determine susceptibility, the prevalence of cefotaxime-susceptible and -intermediately susceptible strains were 81.5 and 18.5%, respectively. However, 3/10 pneumococci that isolated from CSF were penicillin-intermediate susceptible strains. In addition, only 4.9% of the pneumococcal isolates from specimens outside the CNS were intermediately susceptible to cefotaxime. None of the pneumococcal isolates were resistant to cefotaxime. Similar to susceptibility level of cefotaxime, imipenem resistant isolate was not found in this study. Additionally, all of clinical pneumococcal isolates were susceptible to levofloxacin. On the other hand, erythromycin-nonsusceptible pneumococci were 43.5% with 42.4% were in resistant level. Table 13 presented susceptibility of pneumococci in intermediate and high level of resistance which was stratified by specimen source. Table 14 displayed susceptibility of *S. pneumoniae* to selected antibiotics according to site of infection.

Table 13 Pneumococci with intermediate and high level of resistance stratified by specimen source.

Specimen source	Total No. (%) of isolates	No. (%) of isolates resistant to:									
		Penicillin		Cefotaxime		Imipenem		Levofloxacin		Erythromycin	
		I	R	I	R	I	R	I	R	I	R
Blood	27 (29.3)	12 (44.4)	0	0	0	5 (18.5)	0	0	0	0	8 (29.6)
CSF	10 (10.8)	3 (30)	0	0	0	1 (10)	0	0	0	1 (10)	2 (20)
Sputum	55 (59.8)	30 (54.5)	7 (12.7)	4 (7.3)	0	20 (36.4)	0	0	0	0	29 (52.7)

CSF = cerebrospinal fluid

I = intermediate susceptibility, R = resistant

Table 14 Drug-resistant *Streptococcus pneumoniae* stratified by site of infection (pneumonia, bacteremia and meningitis).

Drugs	Pneumonia (*n*=70)				Bacteremia (*n*=12)				Meningitis (*n*=10)			
	S (%)	I(%)	R(%)	I+R	S (%)	I(%)	R(%)	I+R	S (%)	I(%)	R(%)	I+R
Penicillin	35.7	54.3	10	64.3	66.7	33.3	0	33.3	70	30	0	30
Cefotaxime	94.3	5.7	0	5.7	100	0	0	0	100	0	0	0
Imipenem	67.1	32.9	0	32.9	83.3	16.7	0	16.7	90	10	0	10
Levofloxacin	100	0	0	0	100	0	0	0	100	0	0	0
Erythromycin	50	0	50	50	83.3	0	16.7	16.7	70	10	20	30

S= susceptible, I= intermediate susceptibility, R= resistant

4.1.3 MIC Distributions

The MIC distributions of selected antibiotics to pneumococci were shown in Figure 5-9. Penicillin MIC ranged from 0.006 μg/ml to 3 μg/ml. Pneumococcal isolates with penicillin MIC ≥4 μg/ml were not found in this study. MIC_{50} and MIC_{90} of selected antibiotics were presented in Table 15. Table 16 depicted the MICs for pneumococci stratified by specimen source.

Table 15 The MICs for *Streptococcus pneumoniae* to selected antibiotic agents.

	MIC (μg/ml)		
Antibiotic agents	Range	MIC_{50}	MIC_{90}
Penicillin	0.006 - 3	0.19	1.5
Cefotaxime (meningitis)	0.008 - 0.5	0.047	0.5
Cefotaxime (non-meningitis)	0.012 - 2	0.19	1
Imipenem	0.003 - 0.5	0.064	0.25
Levofloxacin	0.038 - 2	0.75	1
Erythromycin	0.023 - 256	0.094	6

Penicillin-susceptible isolates were also found to be 100% susceptible to other tested drugs except erythromycin in which the susceptibility was reduced to 90%. In contrast, penicillin-nonsusceptible pneumococci, particularly highly resistant strains (MIC≥2μg/ml), were also resistant to other β-lactam agents and macrolide. The MIC_{50}/MIC_{90} of pnemococcal isolates susceptible, intermediately susceptible and resistant to penicillin were 0.016/0.032 μg/ml, 0.75/1.5 μg/ml, and 2/2 μg/ml, respectively. The MIC_{50} and MIC_{90} of cefotaxime, imipenem and erythromycin elevated when the MIC_{50} and MIC_{90} of penicillin increased. On the other hand, the MIC_{50} and MIC_{90} of levofloxacin did not relate to MIC_{50} and MIC_{90} of penicillin (Table 17). Table 18 and 19 displayed antimicrobial MIC distributions according to penicillin- and erythromycin-susceptibility for pneumococci, respectively.

Table 16 The MICs for *Streptococcus pneumoniae* stratified by specimen source.

Drugs	MIC (μg/ml)								
	Blood			CSF			Sputum		
	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
Penicillin	0.006-1.5	0.047	1.5	0.006-1.5	0.023	0.38	0.012-3.0	0.5	2.0
Cefotaxime	0.012-1.0	0.032	0.75	0.008-0.5	0.047	0.5	0.012-2.0	0.38	1.0
Imipenem	0.004-0.5	0.064	0.19	0.003-0.25	0.032	0.094	0.004-0.5	0.094	0.25
Levofloxacin	0.25-2.0	0.75	1.5	0.5-2.0	0.75	1.5	0.038-1.5	0.75	1.0
Erythromycin	0.023-16	0.094	4.0	0.032-3.0	0.094	2	0.032-256	2.0	12

CSF = cerebrospinal fluid

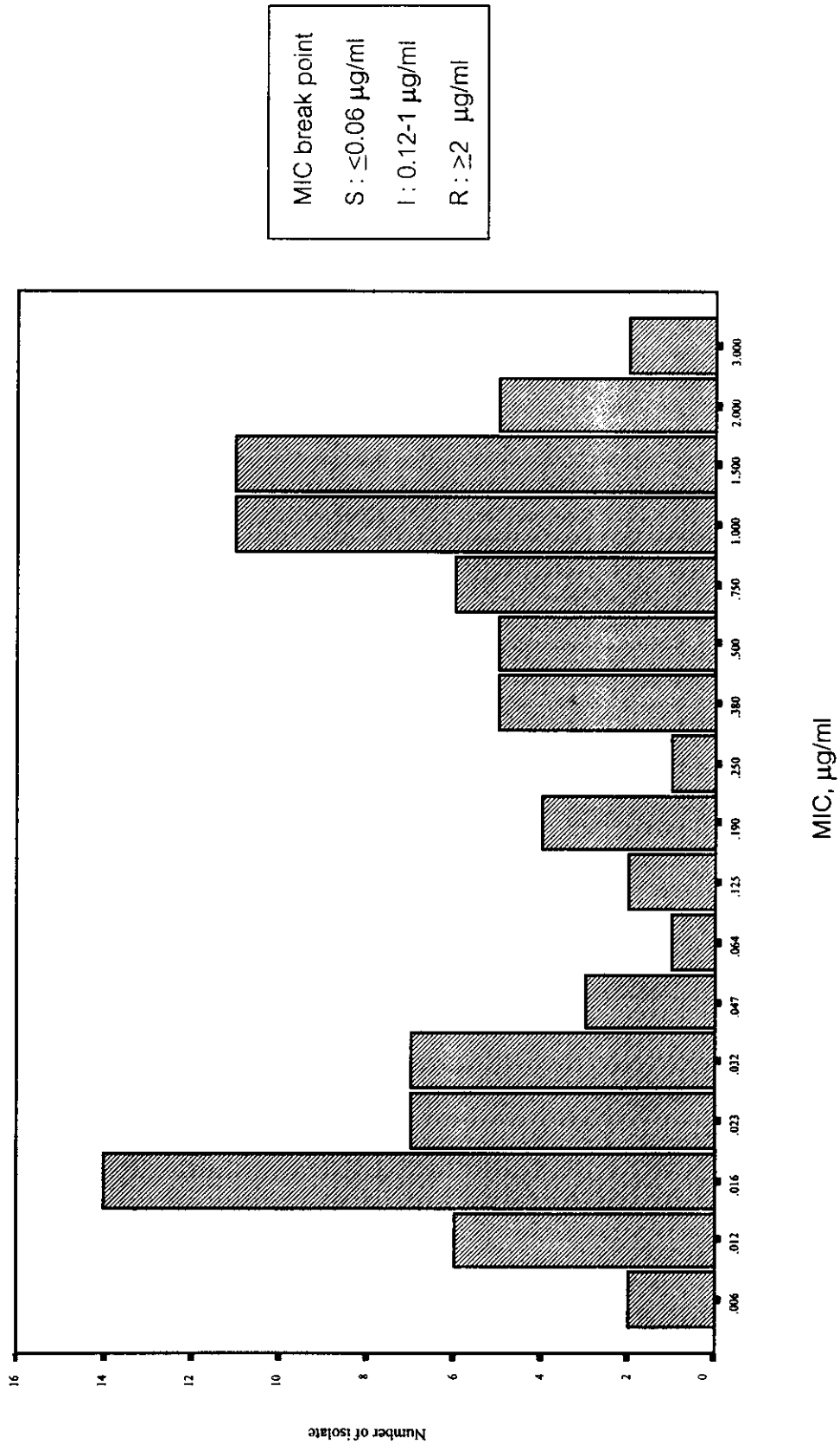

Figure 5 The penicillin MIC for *Streptococcus pneumoniae*.

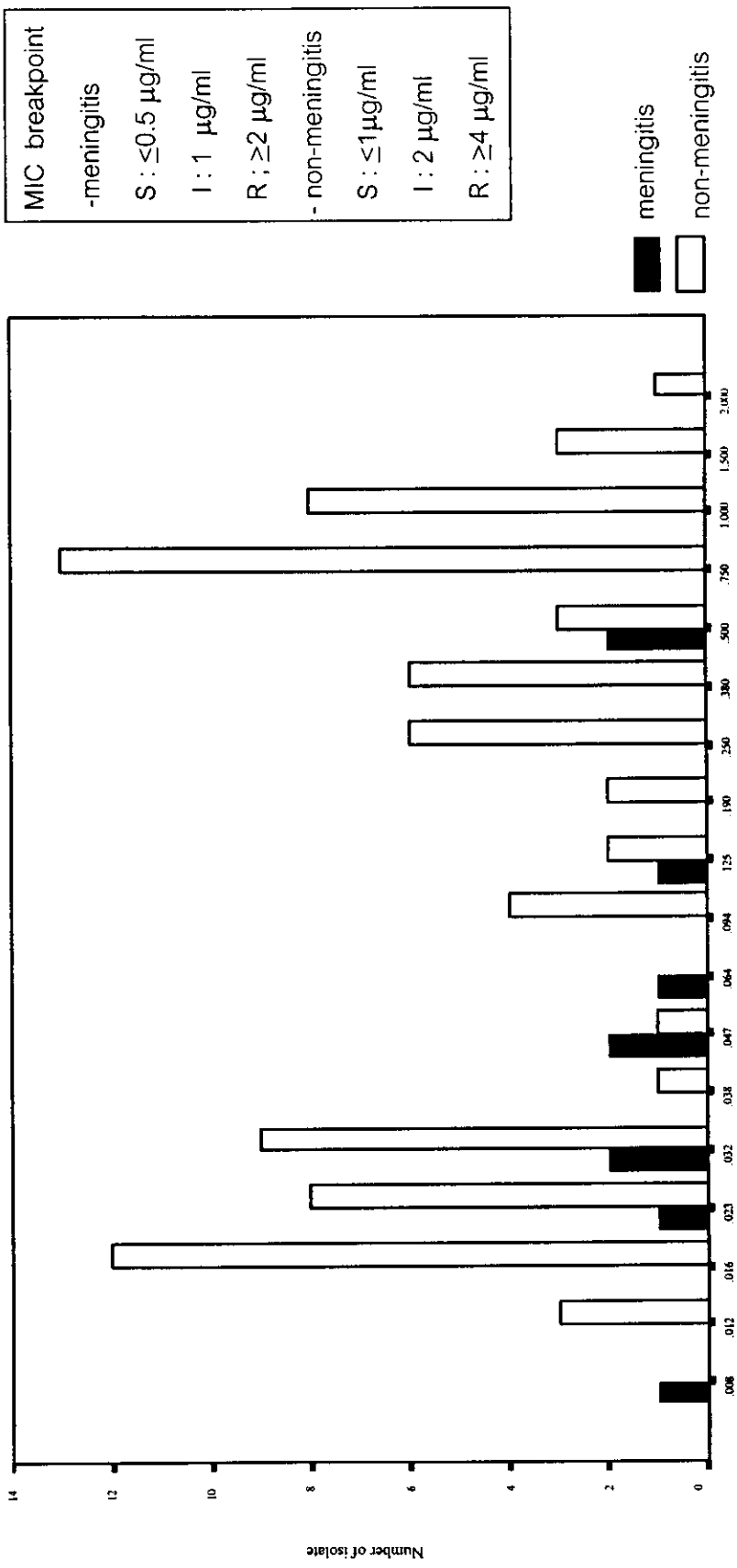

Figure 6 The cefotaxime MIC for *Streptococcus pneumoniae*

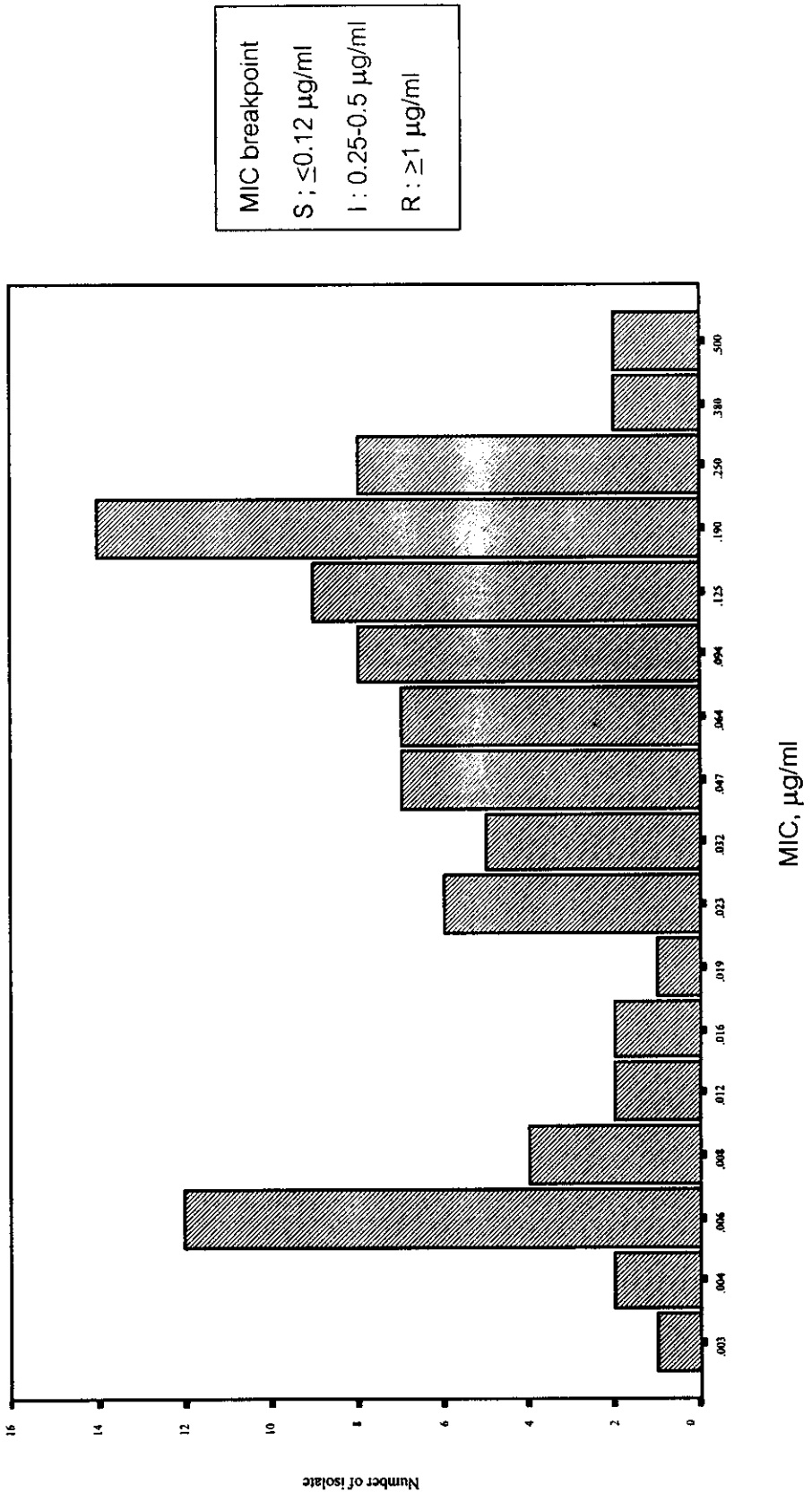

Figure 7 The imipenem MIC for *Streptococcus pneumoniae*.

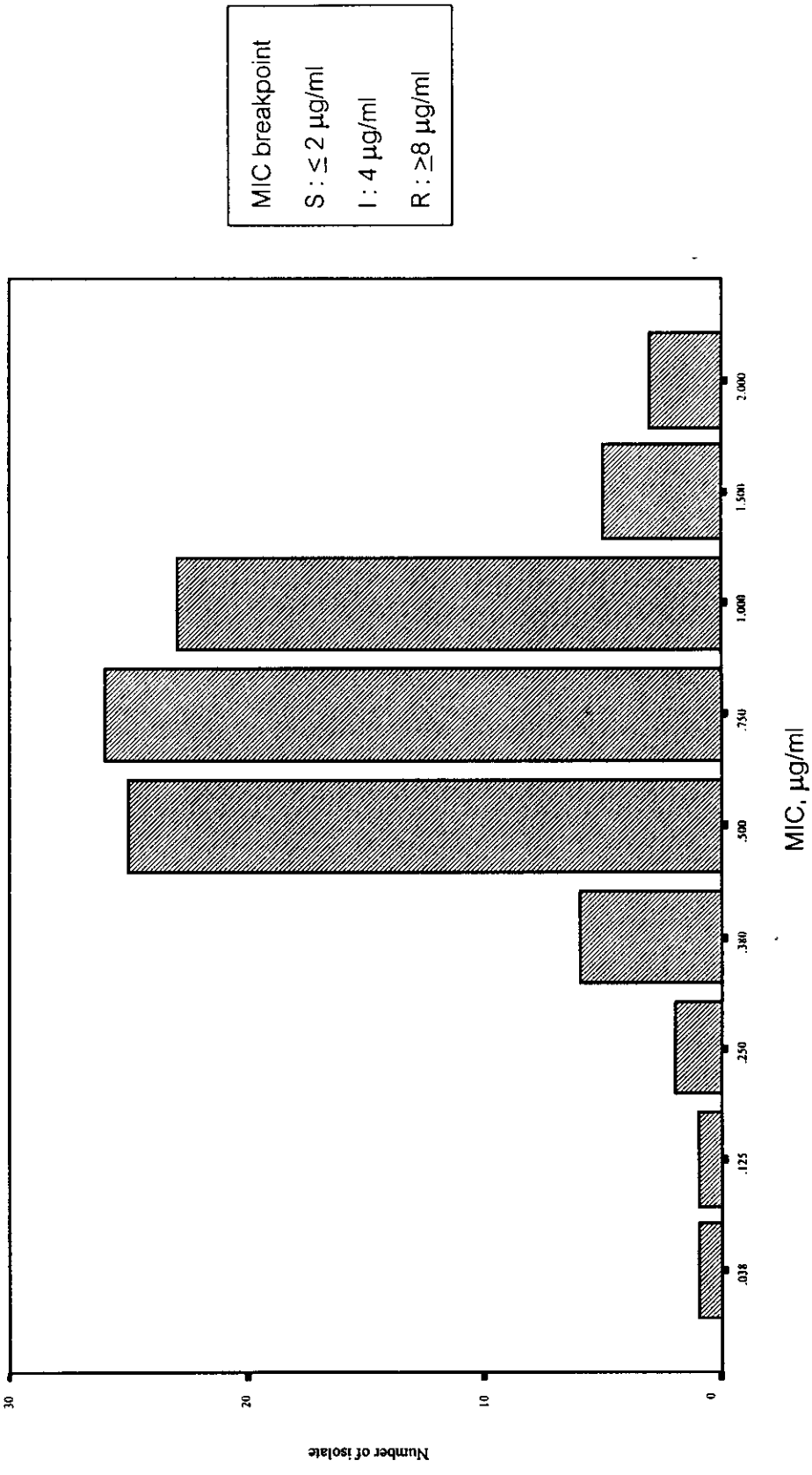

Figure 8 The levofloxacin MIC for *Streptococcus pneumoniae*.

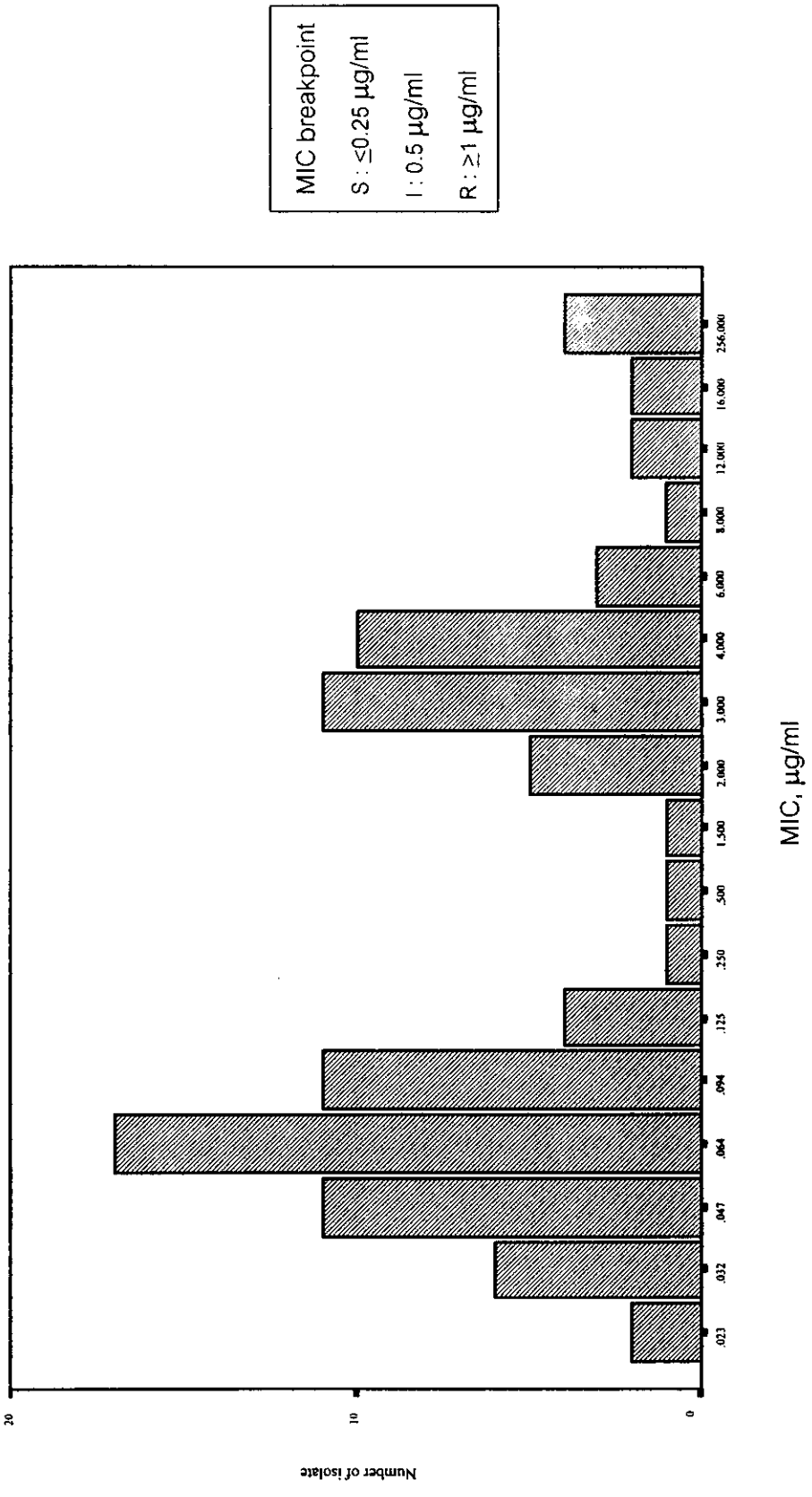

Figure 9 The erythromycin MIC for *Streptococcus pneumoniae*.

Table 17 Susceptibility of penicillin-susceptible and penicillin-nonsusceptible *Streptococcus pneumoniae* to selected antibiotic agents.

	Susceptible to penicillin (*n*=40)			Intermediately susceptible (*n*=45)			Resistant to penicillin (*n*=7)		
	MIC_{50}	MIC_{90}	%susceptible	MIC_{50}	MIC_{90}	%susceptible	MIC_{50}	MIC_{90}	%susceptible
Antibiotic agents	μg/ml			μg/ml			μg/ml		
Cefotaxime (meningitis)*	0.032	0.064	100	0.125	0.5	100	-	-	-
Cefotaxime (non-meningitis)†	0.023	0.032	100	0.38	1	100	1.5	2	42.9
Imipenem	0.012	0.047	100	0.125	0.25	55.6	0.25	0.5	14.3
Levofloxacin	0.75	2	100	0.5	1	100	0.75	0.75	100
Erythromycin	0.064	0.125	90	3	16	33.3	4	256	14.3

* 10 pneumococcal isolates from CNS were calculated, and those of isolates were not resistant to penicillin.

† 82 pneumococcal isolates outside CNS were determined.

NCCLS breakpoint (μg/ml): cefotaxome (meningitis) S=≤0.5, I=1, R=≥2 ; cefotaxime (non-meningitis) S=≤1, I=2, R=≥4 ; imipenem S=≤0.12, I=0.25-0.5, R=≥1 ; levofloxacin S=≤2, I=4, R=≥8 ; erythromycin S=≤0.25, I=0.5, R=≥1

Table 18 Antimicrobial MIC distributions according to penicillin-susceptibility for *S. pneumoniae* (40 penicillin-susceptible isolates, 45 penicillin-intermediate isolates and 7 penicillin-resistant isolates) in Southern Thailand.

Antimicrobial	Percentage of isolates per MIC, µg/ml											
Penicillin	≤0.016	0.023	0.064	0.125	0.25	0.5	0.75	1	2	4		
Penicillin-S	55	17.5	27.5									
Penicillin-I				4.4	11.1	22.2	13.3	24.4	24.4			
Penicillin-R									71.4	28.6		
Cefotaxime	≤0.016	0.023	0.064	0.125	0.25	0.5	0.75	1	2			
Penicillin-S	40	22.5	37.5									
Penicillin-I			2.2	15.6	17.7	24.4	28.9	11.1				
Penicillin-R								42.9	57.1			
Imipenem	≤0.016	0.023	0.064	0.125	0.25	0.5						
Penicillin-S	55	17.5	25	2.5								
Penicillin-I	2.2	0	19.9	33.4	42.2	2.2						
Penicillin-R				14.3	42.9	42.9						
Levofloxacin				0.125	0.25	0.5	0.75	1	2			
Penicillin-S				2.5	2.5	17.5	35	30	12.5			
Penicillin-I					4.4	48.9	20	20	6.6			
Penicillin-R						28.6	42.9	28.6				
Erythromycin		0.023	0.064	0.125	0.25	0.5	0.75	1	2	4	8	16	>256
Penicillin-S		0	62.5	27.5	0	0	0	0	2.5	7.5			
Penicillin-I		4.4	17.7	8.8	2.2	2.2	0	0	8.9	35.6	6.6	6.6	6.7
Penicillin-R									14.3	28.6	14.3	14.3	14.3

S = susceptible, I = intermediately susceptible, R = resistant

Table 19 Antimicrobial MIC distributions according to erythromycin-susceptibility for *S. pneumoniae* (52 erythromycin-susceptible isolates, 1 erythromycin-intermediate isolate and 39 erythromycin-resistant isolates) in Southern Thailand.

Antimicrobial	Percentage of isolates per MIC, μg/ml											
Penicillin	≤0.016	0.023	0.064	0.125	0.25	0.5	0.75	1	2	4		
Erythromycin-S	36.5	13.5	19.2	1.9	7.7	7.6	1.9	5.8	5.7			
Erythromycin-I						100						
Erythromycin-R	7.7	0	2.6	2.6	2.6	12.8	12.8	20.5	33.4	5.1		
Cefotaxime	≤0.016	0.023	0.064	0.125	0.25	0.5	0.75	1	2			
Erythromycin-S	26.9	15.4	26.9	9.6	5.7	7.6	3.8	1.9	1.9			
Erythromycin-I						100						
Erythromycin-R	5.1	2.6	5.2	5.1	12.9	15.4	28.2	17.9	7.7			
Imipenem	≤0.016	0.023	0.064	0.125	0.25	0.5						
Erythromycin-S	38.5	11.5	26.9	11.5	9.6	1.9						
Erythromycin-I				100								
Erythromycin-R	7.7	2.6	12.9	25.6	43.6	7.7						
Levofloxacin				0.125	0.25	0.5	0.75	1	2			
Erythromycin-S				1.9	1.9	17.3	32.7	30.8	15.4			
Erythromycin-I						100						
Erythromycin-R			2.6	0	2.6	53.8	23.1	17.9				
Erythromycin		0.023	0.064	0.125	0.25	0.5	0.75	1	2	4	8	16	>256
Erythromycin-S		3.8	65.4	28.9	1.9								
Erythromycin-I						100							
Erythromycin-R									15.4	53.8	10.3	10.2	10.3

S = susceptible, I = intermediately susceptible, R = resistant

4.2 Part 2: Retrospective Review of Medical Records of Patient Whose Isolates were Obtained in Part 1

Ninety-two medical records of patients with pnemococcal infection were available for reviewing. General characteristic data of studied patients were presented in Table 20. Most of the patients were diagnosed as pneumonia (76.1%), followed by bacteremia (13%) and meningitis (10.9%). In addition, most of pneumococcal isolated from sputum were penicillin-nonsusceptible strains (71.7%). Ages of patients were ranged from 1 day to 105 years with a median of 42.5 years and a mean of 41.5±33.16 years. There was significantly different among patient's age between patient who infected with penicillin-susceptible and -nonsusceptible pneumococci (P=0.011). Due to wide ranging of patients' age, the age was divided into 3 groups: children, adult and elderly. Pneumoncocci isolated from elderly (≥65 years) group was more resistant to penicillin than from other groups. Moreover, among adults (>15 years), patients who were infected with pneumococcal isolates nonsusceptible to penicillin had severity of illness at presentation significantly higher than those patients with penicillin-susceptible pneumococci (P=0.036). Of these patients, underlying diseases were found in 31 patients with penicillin-susceptible pneumococci and 29 patients with penicillin-nonsusceptible strains.

4.2.1 Risk Factors

4.2.1.1 Diseases

Pneumococcal pneumonia were the disease that found penicillin-nonsusceptible strain of pneumococci (45/70) more than pneumococcal bacteremia (4/12) ($P < 0.001$) and pneumococcal meningitis (3/10) (P=0.002). Among the isolates obtained from patients with pneumonia, 50% of the isolates were erythromycin-nonsusceptible strains. After analyzing by multivariate logistic regression, diseases (pneumonia, bacteremia,

and meningitis) was not significant associated with penicillin- or erythromycin-nonsusceptible pneumococci.

Table 20 General characteristics of patients with pneumococcal infection stratified by penicillin susceptibility.

Characteristic	Penicillin susceptibility (%)		Total	*P*-value
	Susceptible	nonsusceptible		
Sex				0.695
Male	23 (57.5)	32 (61.5)	55 (59.8)	
Female	17 (42.5)	20 (38.5)	37 (40.2)	
Age,year (mean±SD)	32.12±30.97	48.45±33.25		0.011*
0-15 years	17 (42.5)	13 (25)	30 (32.6)	0.076
16-64 years	14 (35)	14 (26.9)	28 (30.4)	0.404
≥ 65 years	9 (22.5)	25 (48.1)	34 (37)	0.012*
Clinical specimen				0.029
Sputum	18 (45)	37 (71.1)	55 (59.8)	0.011
Blood	15 (37.5)	12 (23.1)	27 (29.3)	0.132
CSF	7 (17.5)	3 (5.8)	10 (10.9)	0.096†
Diagnosis				
Pneumonia	25 (62.5)	45 (86.5)	70 (76.1)	0.007
Bacteremia	8 (20)	4 (7.7)	12 (13)	0.082
Meningitis	7 (17.5)	3 (5.8)	10 (10.9)	0.096†
Severity of illness‡				
SAPS II score	29.65±11.63	37.86±15.20		0.036*
PRISM score	13.63±11.77	10.54±7.99		0.428*

*The data was analyzed by Mann-Whitney U test.

†CSF and Meningitis were compared by Fisher's exact test.

‡ SAPS II score was used in adults, and PRISM score was used in children.

4.2.1.2 Patients Characteristics

Of 92 patients whose clinical specimens were included in this study, only 79 patients had complete medical record for risk factor analysis. A number of potential variables were evaluated in the study population including ages, prior antibiotic use within 6 months, co-morbidity, co-infection, hospitalization in previous 3 months, and severity of illness at presentation. Multiple logistic regression analysis was completed to determine these variables. Co-morbidity (OR=3.6; 95%CI, 1.1-11.7) was the factor which had statistically significant associated with penicillin-nonsusceptible isolates. In addition, resistant to erythromycin was significant associated with co-morbidity (OR=4.0; 95% CI, 1.2-13.1). However, when analyzed in subgroup of age: children (≤15 years) and adults (>15 years), no variable had significant associated with penicillin- and erythromycin resistance in younger age group. In adult group, after adding other 2 variables (ethanol consumption and smoking) in multiple logistic regression, The variables that had significant associated with penicillin-resistant pneumococci were age (OR=1.050: 95%CI, 1.006-1.096), co-morbidity (OR=8.875; 95%CI, 1.060-74.315), and recent antibiotic use within 6 month (OR=5.468; 95%CI, 1.039-28.766). Erythromycin-resistance also had significant associated with age (OR=1.053; 95%CI, 1.009-1.099) and recent antibiotic use in previous 6 months (OR=11.139; 95%CI, 1.704-72.800) (Table 21). There were no statistically significant between potential variable, including, hospitalization in previous 3 months, ethanol consumption, smoking and severity of illness at presentation and infection caused by drug-resistant pneumococci.

Table 21 Results of multiple logistic analysis of the potential variable for penicillin- and erythromycin-nonsusceptible *Streptococcus pneumoniae* infection in adult.

	Penicillin susceptibility		Erythromycin susceptibility	
	Adjusted Odds ratio (95%, CI)	*P* value	Adjusted Odds ratio (95%, CI)	*P* value
Age, years	1.050 (1.060-1.096)	0.026	1.055 (1.010-1.103)	0.016
Recent hospitalization	4.937(0.742-33.653)	0.103	4.883 (0.688-34.674)	0.113
Co-morbidity	8.875 (1.060-74.315)	0.044	6.521(0.840-50.639)	0.073
Smoking	1.825(0.269-12.408)	0.538	6.602 (0.825-52.813)	0.075
Ethanol consumption	0.978(0.087-11.008)	0.985	0.400 (0.037-4.335)	0.451
Severity at presentation*	1.033(0.974-1.095)	0.279	1.004 (0.946-1.064)	0.903
Prior antibiotic use	5.468 (1.039-28.766)	0.045	11.062 (1.681-72.778)	0.012
Co-infection	0.444 (0.065-3.046)	0.409	0.614 (0.100-3.775)	0.599

* severity at presentation for adult was evaluated by Simplified Acute Physician Score II (SAPS II) score.

4.2.2 Patient Outcome

All 92 patients, all patients had community-acquired pneumococcal infection. Eighty-eight patients came from home and 4 patients were referred from smaller hospitals after admitting for 1 or 2 days. Pneumonia was the most common diagnosis followed by bacteremia and meningitis. Susceptibilities of pneumococcal isolates from patients with pneumonia, bacteremia and meningitis were displayed in Table 14 in previous section (4.1.2). More than half of the patients (65.2%) had co-morbidity, and chronic lung disease (asthma and COPD) was the most common disease. A mean length of hospital stay of the studied group was 9.5±9.19 days (range, 1-60 days). All of 5 pneumococcal isolates from patients with HIV infection were resistant to penicillin. One of those patients had AIDS (Table 22).

Table 22 Susceptibility of *Streptococcus pneumoniae* to penicillin stratified by co-morbidity

	Penicillin susceptibility		*P* value
	Susceptible (*n*=40)	Nonsusceptible (*n*=52)	
Diabetes	1 (2.5)	2 (3.8)	1.000*
Chronic lung disease	8 (20)	15 (28.8)	0.331
AIDS and HIV infection	0	5 (9.6)	0.066*
Renal disease	1 (2.5)	1 (1.9)	1.000*
Hepatic disease	1 (2.5)	3 (5.8)	0.630*
Malignancy	3 (7.5)	3 (5.8)	1.000*
Leukopenia	1 (2.5)	4 (7.7)	0.383*
Coronary artery disease	6 (15)	7 (13.5)	0.834
Hypertension	2	8	0.177*
Co-infection	10	11	0.803

* Fisher's exact test

A favorable response within 72 hours of antibiotic therapy was observed in 40 (43.5%) patients. There were 55% (22/40) of patients with penicillin-susceptible infection compared with 33.3% (15/45) of patients with penicillin-intermediate susceptibility isolates (P=0.09) and 42.8% (3/7) of patients who were infected with penicillin resistant strains (P=0.68). Overall, successful final clinical outcome was found in 57 (62%) patients (25 patients with penicillin-susceptible pneumococci and 32 patients with penicillin-nonsusceptible strains). Nineteen (20.7%) patients died within 7 days after positive culture of pneumococci, but only 15/92 (16.3%) deaths related to pneumococcal infection. None of patients who infected with pneumococci resistant to penicillin (MIC≥2 μg/ml) died. However, clinical outcome of 17/92 (18.5%) patients was undetermined due to premature discontinuation of antibiotic requested by patients or relatives (9 patients with pneumonia, 3 patients with bacteremia and 5 patients with meningitis). Among patients who received β-lactam antibiotic for treatment of pneumonia and bacteremia, there was no significant difference between penicillin-susceptible and -nonsusceptible pneumococcal infection in term of length of stay, admission to ICU, severity of illness score (SAPS II and PRISM score), favorable response within 3 days, day of fever resolution, final clinical outcome and death (Table 23). Despite of higher scores of severity (SAPS II score) at presentation among adult patients were infected with penicillin–nonsusceptible strains, final clinical outcome was not different between patient with penicillin-susceptible and -nonsusceptible pneumococci. Moreover, all of patients whose infection caused by cefotaxime-nonsusceptible strains had successful final clinical outcome.

Table 23 Comparison of outcome difference between penicillin-susceptible and -nonsusceptible infection among patient who received β-lactam therapy.

	Penicillin susceptibility		*P* value
	Susceptible	nonsusceptible	
Length of stay (mean±SD)	9.75±5.66	10.77±11.68	0.658*
Admission to ICU	8	8	0.562
Severity at presentation			
SAPS II (mean±SD)	29.20±11.92	37.39±15.29	0.057†
PRISM (mean±SD)	15.46±12.10	9.91±8.54	0.257†
Favorable response at 72 hours	19	16	0.135
Day of fever resolution (mean±SD)	4.52±3.50	5.45±4.02	0.374*
Final clinical outcome			0.641
Successful	23	28	
Failure	5	10	
Undetermined	6	6	
Death	2	9	0.077‡

* Student's *t*-test

† Mann Whitney U test

‡ Fisher's exact test

4.2.2.1 Pneumonia

Of 92 patients with pneumococcal infection, 70 patients had documented pneumonia. Most of patients infected with non-susceptible strains of pneumococci, 65% (45/70). There was not different between patients with pneumonia who were infected with penicillin-susceptible and –nonsusceptible pneumococci in term of admission to ICU, co-infections, death related to pneumococcal infection, and final clinical outcome. In contrast, 24/70 patients with penicillin-nonsusceptible strains had unfavorable response within 72 hours more than those patients with penicillin-susceptible strains (8/70) (P=0.036). Fifteen patients had bacteremic pneumonia. The blood culture of eight patients grew penicillin-nonsusceptible pneumococci. Admission to ICU, co-infections, favorable response within 72 hours, death related with pneumococcal infection, and final clinical outcome of patients infected with penicillin-nonsusceptible pneumococcal pneumonia did not differ significantly from those with penicillin-susceptible strains. We also stratified the clinical outcome by age group: children (≤15 years), adults (16-64 years) and elderly (≥65 years) for pneumonia. Clinical characteristics, pattern of antibiotic use and clinical outcome of patients with penicillin-susceptible and –nonsusceptible pneumococci were displayed in Table 24 according to age group.

Table 24 Characteristics and outcomes of patients with pneumococcal pneumonia stratified by penicillin susceptibility.

	Penicillin-susceptible pneumococci			Penicillin-nonsusceptible pneumococci		
	Children (*n*=7)	Adults (*n*=11)	Elderly (*n*=7)	Children (*n*=11)	Adults (*n*=10)	Elderly (*n*=24)
Characteristics						
Sex (male : female)	2:5	6:5	5:2	7:4	6:4	15:9
Age, year (mean±SD)	5.16±5.30	40.73±14.20	78.43±14	2.49±3.08	35.40±13.76	78.42±9.45
Length of stay, day (mean±SD)	9.71±8.06	8.36±4.76	6.71±4.11	7.55±6.65	6.30±5.95	14.17±14.22
Underlying disease	5	11	6	5	3	18
Initial antibiotic therapy						
Penicillin or Ampicillin	4	5	2	8	5	9
Cephalosporin	2	5	3	3	4	11
Fluoroquinolone	0	1	1	0	0	1
Macrolide	0	0	0	0	1	1
Combination therapy	0	0	1	0	0	2
Final clinical outcome						
Success	5	7	7	8	5	16
Failure	1	2	0	2	3	5
Undetermined	1	2	0	1	2	3
Death (relevant / irrelevant)	0/1	1/1	0	1/0	3/0	5/1

Eighteen children (9 male, 9 female) with mean age of 3.53 (± 0.98; range, 0.1-14) years had pneumococcal pneumonia. 10/18 (55.6%) patients had underlying disease: 2 patients with HIV infection, 3 patients with malignancy and 5 patients with other chronic underlying disease. Of these children, 17/18 patients received antibiotic for at least 48 hours. Antibiotics were prescribed to children with pneumonia as follow: 1 child received oral amoxicillin alone, 11 children received parenteral penicillin or equivalent β-lactam antibiotic (ampicillin), and 5 children received third generation cephalosporin. One patient did not receive antibiotic therapy and had spontaneous clinical improvement in days3. A favorable response within 72 hours was observed in 58.8% (10/17) of patients and 3/17 (17.6%) patients responded to antibiotic therapy within 7 days. There were 2 patients died, but only 1 patient died from pneumococcal pneumonia. Moreover, 2 patients who were infected with penicillin-intermediate susceptibility strains experienced treatment failure. One patient failed to respond to cefotaxime after 7 days of therapy; vancomycin was subsequently added for 2 days. Another patient's clinical status had not changed after receiving ampicillin for 11 days. This patient was subsequently treated with ceftriaxone for 7 days. However, both patients' final clinical outcome could not be determined because patients' parents denied therapy and patients were discharged with unimproved clinical status. In addition, two patients were infected with multidrug-resistant strains, one responded to ampicillin within 3 days and another patient's clinical outcome could not be assessed because of denial of therapy. A mean day of fever resolution among patient with penicillin-susceptible pneumococci (5.75±3.77 days) was not significantly different to those with penicillin-nonsusceptible isolates (3.56±1.42 days). According to drug-susceptible versus -nonsusceptible pneumoccocal pneumonia, there was not significant different in term of length of hospitalization, favorable response within 72 hours, favorable response within 7 days, final clinical outcome and death between 2 groups. On the other hand, this study found that pediatric patients who were infected with penicillin susceptible pneumococcal isolates required more ICU admission than those who infected with nonsusceptible strains (P=0.011). However, after adjustment the

severity at presentation and co-infection by multiple logistic regression, penicillin susceptibility did not associated with ICU admission.

Those of adult group, 14/21 (66.7%) patients had underlying disease as followed: 1 with malignancy, 1 with CAD, 1 with SLE, 1 with liver disease, 2 HIV infection, 6 with COPD, and 2 with other co-morbidity. A mean length of hospitalization among patients who infected with penicillin-susceptible and -nonsusceptible pneumococci pneumonia were 8.36±4.76 and 6.30±5.59 days, respectively. Day of fever absence ranged from 2-5 days with a mean of 2.86±1.07 days among patients infected with penicillin-susceptible pneumococci and 3.33±1.53 days among patients infected with penicillin-nonsusceptible strains. In this group, 10/21 (47.6%) patients received penicillin or ampicillin for at least 48 hours. 9/21 (42.8%) patients received cephalosporin (one of these received fourth generation cephalosporin, cefipime). Furthermore, fluoroquinolone and macrolide were prescribed to 2 patients. A favorable response within 72 hours was found in 11/21 (52.4%) patients, and a favorable response within 7 days was found in 2/21 (9.5%) patients. Five (23.8%) patients died, but one died irrelevantly to pneumococcal pneumonia. Clinical outcome of 3 patients was not determined; 2 of these patients denied therapy and were discharged with worse status, and one patient was referred to local hospital with unimproved clinical status. Treatment failure was found in 5 mortality cases. 3 of these 5 patients with penicillin resistant strains received high dose penicillin (2/3) and cefotaxime (1/3). Two patients were infected with penicillin susceptible strains; one received oral gatifloxacin alone and another patient was treated with cefotaxime. There were 7 patients with multidrug-resistant isolates, one of these patients who infected with pneumococci resistant to 4 antibiotic agents responded to ampicillin within 3 days. Three of these patients died; one denied therapy and was discharged with worse status. Similar to children group, there was no significantly difference between patients who infected with drug-suscpetible pneumococci and those with drug-nonsusceptible pneumococci in term of length of hospital stay, day of fever

absence, admission to ICU, favorable response within 3 and 7 days, final clinical outcome and death.

Forty four percent (31/70) of pneumococcal pneumonia were elderly. Almost those patients (24/31) had co-morbidity: 7 patients with immunosuppresion disease (malignancy, ESRD, liver disease, SLE, and leukopenia), 10 patients had 2 underlying diseases and 2 patients had 3 underlying diseases. In this group, penicillin was used in 11 patients, followed by non-pseudomonal third generation cephalosporin, 11; cefazolin, 3; fluoroquinolone, 2; and macrolide, 1. Three patients received combination therapy of penicillin and cefotaxime or ceftriaxone. A comparison of the favorable response within 72 hours of patients with infection caused by pneumococci susceptible to penicillin versus those with isolates of pneumococci that were not susceptible to penicillin demonstrated a significant difference between two groups (P=0.023). Patients with penicillin-nonsusceptible strains had more unfavorable response to antibiotic therapy within 72 hours than those who infected with penicillin-susceptible strains. However, neither final clinical outcome nor a mean day of fever resolution (2.67$\pm$0.82 days among patients whose strains were susceptible to penicillin VS 3.56$\pm$1.42 days among patients whose strains were nonsusceptible to penicillin) had significant associated with penicillin susceptibility. Ten patients had multidrug-resistant pneumococci pneumonia: 4 patients responded to third generation cephalosporin; one responded to cefazolin and one had favorable response within 3 day after receiving oral amoxicillin. Moreover, one of these patients with pneumococcal isolate resistant to 4 antibiotic agents had favorable response to ceftriaxone within 7 days. However, six patients who infected with penicillin-nonsusceptible pneumococci died, but one died due to underlying disease. Four of mortality cases died due to pneumococcal pneumonia with septicemia after receiving ceftriaxone (2/4), penicillin (1/4), and penicillin + cefotaxime (1/4) for 2 days, moreover, two of these patients was infected with multidrug-resistant strains. One patient with COPD who received ceftriaxone for 2 days died from respiratory failure. Clinical outcome of 3 patients could not be evaluated since 2 patients had hospital-acquired

infection after long duration of hospitalization and one patient denied therapy. Similar to two groups above, there was no statistically significant difference between patient infected with drug-susceptible and -nonsusceptible pneumococci in term of length of hospital stay, admission to ICU, response within 7 days, and death.

4.2.2.2 Bacteremia

Twelve isolates of pneumococci were from the blood of patients without specific site of infection. All of these isolates were susceptible to cefotaxime and levofloxacin. On the other hand, 4 pneumococcal isolates were intermediately susceptible to penicillin. Nine (75%) patients had underlying condition: 1 with AIDS, 1 with liver disease, and 7 with other chronic diseases. Five patients were treated with penicillin and 5 patients received cephalosporin, one of these patients received cefazolin. Two patients received combination therapy: third generation cephalosporins + macrolides and third generation cephalosporins + penicillins. Antibiotic pattern and clinical outcome of patients with pneumococcal bacteremia were shown in Table 25. There was one patient infected with multidrug-resistant, however, he had favorable response to ceftriaxone within 72 hours. Similar to pneumococcal pneumonia, there was no significantly different between patients who infected with drug-susceptible strains versus those with drug-nonsusceptible strains in term of ICU admission, length of hospitalization, day of fever absence, clinical outcome and death.

Table 25 Antibiotic administered and clinical outcome of patient with pneumococcal bacteremia.

Patient No.	Patient age	Resistance	Penicillin MIC,(μg/ml)	Antibiotic therapy	Outcome
1	1 day	None	0.006	Ampicillin	Improved within 3 days
2	1 day	None	0.047	Ampicillin followed by Cefotaxime	Died in day 3
3	3 years	None	0.032	Ampicillin	Improved within 3 days
4	4 years	None	0.047	Cefotaxime	Improved within 7 days
5	4 years	None	0.032	Ampicillin + cefotaxime followed by Penicillin	Undetermined, denied Tx
6	9 years	Pen, Imi, Ery	1.50	Ceftriaxone + erythromycin	Improved within 3 day
7	43 years	Pen, Imi	1.50	Ceftriaxone	Undetermined, denied Tx
8	45 years	None	0.023	Ceftriaxone	Died in day 4
9	58 years	Pen, Ery	0.75	Ceftriaxone	Died in day 2
10	66 years	None	0.016	Ampicillin followed by ceftriaxone+Penicillin	Improved more than 14 days
11	80 years	None	0.016	Penicillin	Improved withinday2
12	89 years	Pen	0.19	Cefazolin	Improved within 14 days

Tx. = therapy , Ery = erythromycin, Imi = imipenem, Pen = penicillin

4.2.2.3 Meningitis

Ten patients with pneumococcal meningitis were included, 6/10 patients were children and 4/10 patients were adults. All pneumococcal isolates from these patients were susceptible to cefotaxime and levofloxacin. Penicillin-intermediate susceptibility was found in 3 isolates. Empirical antibiotic therapy was prescribed as third generation cephalosporin (7/10), combination between third generation cephalosporin + penicillin (2/10). One child whose isolate was multidrug-resistant (penicillin, erythromycin and imipenem) pneumococci died after receiving high dose cefotaxime for 2 days. One patient with ambiguous clinical presentation did not receive antibiotic treatment and died later. The mean length of hospital stay and day of fever resolution were 9.40$\pm$6.24 and 6.17$\pm$3.06 days, respectively. Table 26 illustrated antibiotic pattern and clinical outcome of patients with pneumococcal meningitis.

Table 26 Antibiotic pattern and clinical outcome of patient with pneumococcal meningitis.

Patient No.	Patient age	Resistance	Pen/CTX MIC,(μg/ml)	Antibiotic therapy	Outcome
1	2 months	None	0.006/0.008	Cefotaxime, 7 days	Undetermined, denied Tx
2	3 months	None	0.016/0.047	Cefotaxime, 5 days	Undetermined, denied Tx
3	5 months	None	0.064/0.064	Cefotaxime, 11 days followed by Ceftazidime	Died in day 22, hospital acquired infection
4	6 months	Pen, Imi, Ery	1.5/0.5	Cefotaxime, 2 days	Died in day 2
5	11 months	Ery	0.012/0.023	Cefotaxime, 14 days	Improved within 14 days
6	4 years	None	0.023/0.032	Cefotaxime, 5 days followed by Penicillin, 10 days	Improved within 3 days
7	18 years	None	0.023/0.047	Cefotaxime, 7 days	Improved within 3 days
8	26 years	None	0.023/0.032	Ceftriaxone, 9 days	Undetermined, denied Tx
9	43 years	Pen, Ery	0.38/0.5	Ceftriaxone, 14 days	Improved within 7 days

Ery = erythromycin, Imi = imipenem, Pen = penicillin

Tx = therapy

4.2.3 Microbiological Outcome

Organism culture was repeated after antibiotic treatment in 20 cases. Of these 20 cases, 16 pneumococcal isolates were from patients with pneumonia, 2 isolates were from patients with bacteremia, and 2 isolates were from patients with meningitis. Penicillin-susceptible strains were found in 10 isolates and the other 10 isolates were penicillin-intermediately susceptible strains. All of isolates were susceptible to cefotaxime and levofloxacin. Susceptibility to imipenem and erythromycin showed as followed: 17/20 isolates were susceptible to imipenem; 3/20 isolates were imipenem-intermediate susceptible; 13/20 isolates were erythromycin susceptible strains; and 7/20 isolates were resistant to erythromycin. Microbiological outcome were assessed in all 20 patients, the documents showed that all of *S. pneumoniae* were eradicated after antibiotic therapy. Table 27 presented pattern of antibiotic use and clinical outcome of patients with eradicated microbiological outcome.

Table 27 Clinical outcome and pattern antibiotic use of patients whose microbiological outcome was eradicated.

Patient No.	Resistance	Antibiotic therapy	Outcome
1	None	AMP followed by PGS +CTX	Improved more than 14 days
2	Pen	LFX follow by Amoxiclav. and CTX	Improved more than 14 days
3	Pen	CTX followed by PGS and Imi	Improved more than 14 days
4	None	CTX	Improved within 72 hours
5	Pen, Ery	PGS followed by GTX	Improved within 7 days
6	Pen, Ery	CTX followed by VMC	Undetermined, denied Tx.
7	Pen, Ery	CTX	Improved within 7 days
8	Pen, Imi	CTX followed by PGS, AMG, CPZ	Died from hospital-acquired infection
9	None	CTX	Improved within 72 hours
10	Ery	PGS followed by AMG	Died from hospital-acquired infection
11	None	PGS followed by CTX and Imi	Undetermined, denied Tx.
12	Pen, Ery	Oral amoxicillin	Improved within 72 hours
13	None	CTX	Improved within 72 hours
14	Pen, Ery	PGS followed by CTX	Undetermined, denied Tx.
15	Pen, Imi. Ery	AMP followed by AMG+CTX	Undetermined, denied Tx.
16	None	CTX+AMG	Improved within 7 days
17	None	CTX+AMG	Undetermined, denied Tx.
18	Pen, Imi	AMP	Improved within 72 hours
19	None	CTX followed by PGS	Improved within 72 hours
20	None	AMP	Improved within 72 hours

Tx. = therapy, Ery = erythromycin, Imi = imipenem, Pen = penicillin, PGS = penicillin G sodium, CTX = cefotaxime or ceftriaxone, LFX = levofloxacin, Amoxiclav = amoxicillin+clavolanate, GTX = gatifloxacin, VMC = vancomycin, AMG = amikacin or gentamicin, CPS = cefoperazone