# CHAPTER 2

# REVIEW OF LITERATURES

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## 2.1 *Streptococcus pneumoniae*

**Figure 1** *Streptococcus pneumoniae* replicates in pair and lancet-shaped.

The pneumococci (*Streptococcus pneumoniae*) are gram-positive diplococci, which often manifest lancet-shaped and pair or chain. This organism grows best on complex media containing additive; for example blood or serum, in an atmosphere 5-10% $CO_2$. The organism produces a green halo during growth on blood agar due to producing pneumolysin (α-hemolysin). Therefore, identification of pneumococci in the microbiological laboratory is determined by four reactions: 1) α-hemolysis of blood agar; pneumococci produce α-hemolysin that breaks down hemoglobin into a green pigment. 2) catalase negative 3) susceptibility to optochin 4) solubility in bile salts (Harwell and Brown, 2000; Musher, 2000; Brooks, Butel and Morse, 2001).

Almost clinical isolates of pneumococci contain an external polysaccharide capsule. Pneumococcal isolates that producing a lot of capsules appear as large mucoid colonies. Thus, ninety serotypes of pneumococci have been identified on the basis of antigenic difference in their polysaccharides. There are 2 systems for numbering pneumococcal serotypes. Firstly, the American numbering system numbers from 1 to 90 in the order which they were identified. Secondly, the Danish numbering system, more widely accepted, groups serotypes according to antigenic similarities; for instance, 6A, 6B, 19A, 19F. Serotype difference represents an important virulence factor which can be avoided from immune detection by antibodies and phagocytosis. Infected with pneumococci is relatively common in newborn up to 2 years of age and in adults older than 65 years. Serotypes of 1 to 8 are the usual causing pneumonia, bacteremia, and meningitis in adult. Additionally, serotype 6, 14, 19, and 23 are frequently the cause of pneumococcal infection in children. More than 80% of resistant isolates are 6A, 6B, 9V, 14, and 23F. 23F and 9V, and these are multidrug-resistant that are found in the worldwide (Hall, 1998; Chenoweth, *et al*, 2000; Harwell and Brown, 2000; Musher, 2000). In Asia, Song and colleagues (1999) found that serotype 23F and 19F were the most common resistant serotypes. In Thai children lower than 5 years of age with acute respiratory tract infection, Sunakorn (1996) established that serotype 6 was the most common serotype followed by 12, 15, 19, and 14, respectively. Besides producing

polysaccharide capsule, pneumococci produce IgA protease which helps the organism adhere to mucosa without any symptom. If mucociliary clearance is impaired, colonization will rapidly replicate and invade to the host to cause clinical infection (Harwell and Brown, 2000; Musher, 2000).

## 2.2 Definition of Drug-Resistant *Streptococcus pneumoniae*

### 2.2.1 Penicillin-Resistant *Streptococcus pneumoniae*

Penicillin G was highly effective drug in the past. It was the drug of choice for pneumococcal infection, including potentially lethal pneumonia, bacteremia, and meningitis. Susceptibility of pneumococci to penicillin is defined into 3 general categories: susceptible, intermediately susceptible, and resistant. These are based on the level of minimum inhibitory concentrations (MIC). The strains with MIC of penicillin are less than or equal 0.06 μg/ml will be classified as penicillin-susceptible pneumococci. Relatively penicillin-nonsusceptible pneumococcal isolates with penicillin MIC are range from 0.1-1.0 μg/ml are classified as intermediately susceptible, and with penicillin MIC are more than or equal 2.0 μg/ml are defined in term truly resistance (Friedland and McCracken, 1994; Barry, 1999; Chenoweth, *et al*, 2000; NCCLS, 2002).

### 2.2.2 Other Drug-Resistant *Streptococcus pneumoniae*

The resistance to other antibiotics increases in parallel with penicillin resistance. Similar to susceptibility of pneumococcal isolates to penicillin, classification of susceptibility for other antimicrobial has been categorized into 3 categories: susceptible, intermediately susceptible and resistant (Table 1) (Barry, 1999; Chenoweth, *et al*, 2000; NCCLS, 2002).

### 2.2.3 Multidrug-Resistant *Streptococcus pneumoniae*

Multidrug-resistant pneumococcal isolate was first reported from South Africa in 1970s, and now many countries are endemic areas for this strain. Pneumococci that resist to 3 or more antimicrobials of different classes (penicillins, cephalosporins, macrolides, fluoroquinolones, co-trimoxzole, or carbapenems) are defined as multidrug-resistance (Crook and Spratt, 1998; Chenoweth, *et al*, 2000; Harwell and Brown, 2000).

**Table 1** NCCLS breakpoints for interpretation of MICs for *Streptococcus pneumoniae*

| Antibiotics | MIC (μm/ml) | | |
|---|---|---|---|
| | Susceptible | Intermediate | Resistant |
| Penicillin | ≤ 0.06 | 0.12-1 | ≥ 2 |
| Cefotaxime (meningitis) | ≤ 0.5 | 1 | ≥ 2 |
| Cefotaxime (non-meningitis) | ≤ 1 | 2 | ≥ 4 |
| Levofloxacin | ≤ 2 | 4 | ≥ 8 |
| Imipenem | ≤ 0.12 | 0.25-0.5 | ≥ 1 |
| Erythromycin | ≤0.25 | 0.5 | ≥ 1 |

Ref. : NCCLS guideline, 2002.

## 2.3 Mechanisms of Antimicrobial Resistance

The major mechanisms of antimicrobial resistance are classified into 4 categories as shown in Figure 2. First, specific enzymes producing from organism may inactivate or modify the drug before or after it enters the bacterial cell. Second, bacterial envelope may be modified and contributed to less penetration of antimicrobial into bacterial cell. Third, drug may be expelled from bacterial cell. The last is target modification of bacterial cell (Sefton, 2002).

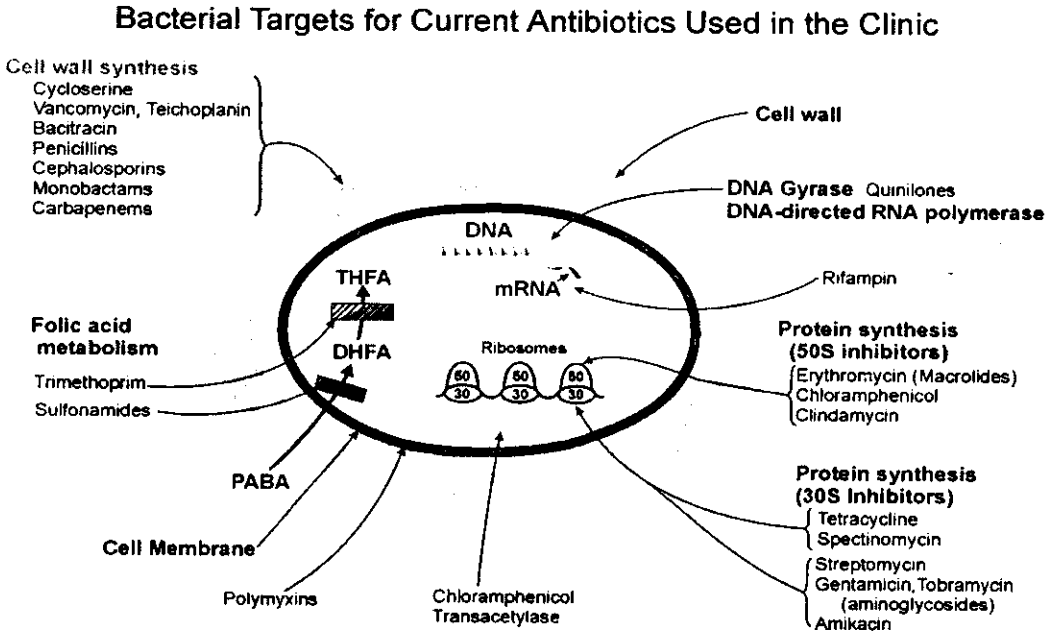
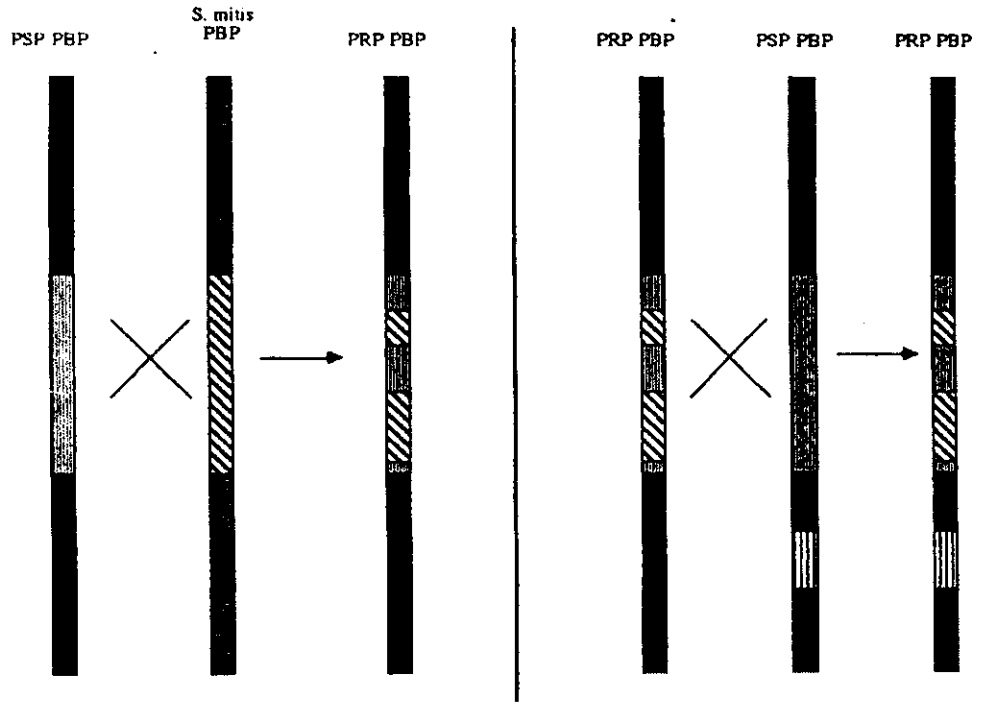



Figure 2 Targets, mode of action and mechanisms of resistance of the major classes of antibacterial drugs.

### 2.3.1 Pneumococci Resistant to β-lactam Antibiotics

*Streptococcus pneumoniae* is the one of few bacteria species that remains unknown about the expression of β-lactamase. Resistance to β-lactam antibiotics is due to changing of one or more penicillin-biding proteins (PBPs), which decrease the PBPs affinity for penicillin and other β-lactam antibiotics. Pneumococci have five high-molecular weight PBPs that are thought to be the target molecules of action of β-lactam antibiotics. Genetic experiments have shown that pneumococcal resistant to penicillin in clinical isolates acquire low affinity variants of these PBPs (PBP 1a, 2b, and 2x). Similar evidence has been shown that high level resistant to extended-spectrum cephalosporin is due to the acquisition of low-affinity PBP 1a and 2x. In addition, the other publications have been shown that there were the horizontal spread of the PBPs genes in the resistant pneumococcal isolates to *Streptococcus oralis* and the viridans streptococci, well known as "mosaic nature". The mosaicism, natural transformation of segmented PBPs genes of more resistant viridans streptococci to colonized pneumococcal strains

in pharyngeal, leads to high level penicillin resistance (Figure 3). PBPs which encode by mosaic PBP genes present a reduced affinity for all β-lactam antibiotics, including cephalosporins and carbapenems (Bonafede and Rice, 1997; Tomasz, 1997; Crook and Spratt, 1998; Maiden, 1998; Jacob, 1999).




Individual stick drawings represent PBPs from *S.mitis*, penicillin-sensitive *S.pneumoniae* (PSP) and penicillin-resistant *S.pneumoniae* (PRP). The *left* side of the figure represents the recombination of heterologous PBP genes, which, given the need to cross over across relatively narrow regions of homology and create a functional and resistant PBP gene, would be expected to be a rare event. The *right* side of the figure represents a conception of subsequent recombination events between a PBP gene from a resistant pneumococcus and one from a susceptible strain. Given the extensive homology that would be expected between the two genes, a crossover event between DNA from two members of the same species would be expected to be more frequent than the original event leading to the resistant PBP. Such a mechanism could explain the

observation of increasing diversity of PBP serotypes as the underlying prevalence of PRP within a population increases (Bonafede and Rice, 1997).

**Figure** 3 Schematic representation of mechanism of penicillin-resistance in *Streptococcus pneumoniae.*

### 2.3.2 Pneumococci Resistant to Macrolides

Macrolide resistance is entirely due to 2 mediators. Firstly, *mef*E gene or so-called M Phenotype, a gene encoding an efflux pump which provided resistance to 14- and 15-membered macrolides only (Figure 4). The second is *erm*AM gene, a 23 rRNA methylase contributing to macrolide (M)-, lincosamide (e.g.clindamycin) (L)- and streptogramin B ($S_B$)-resistance ($MLS_B$ phenotype). In the North America, most macrolide-resistant isolates bear M phenotype, particularly in children younger than 5 years (Kamradt, *et al*, 2000; Hyde, *et al*, 2001). Majority of erythromycin-resistant pneumococci carried the *mef*A genes were found in Hong Kong (Margaret, *et al*, 2001). On the other hand, most pneumococcal isolate that resisted to macrolide was due to a mutation in *erm*B in Europe (Table 2) (Tait-Kamrade, *et al*, 2000), and *erm*A was described in Greece (Lynch III and Martinez, 2002).

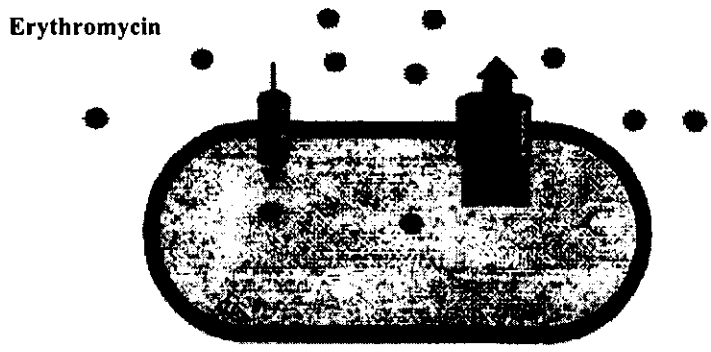



**Figure 4** Efflux pump, bacteria manufacture protein pumps that pump the antibiotic out so that it does not accumulate to a high enough internal concentration to block protein synthesis.

Table 2 Mechanisms for pneumococcal resistance to macrolides

| Target site (ribosomal) mutation | Efflux pump modification |
|---|---|
| $MLS_B$ phenotype | M phenotype |
| Erythromycin ribosomal methylation (*erm*) gene | Macrolide efflux (*mef*E) gene |
| Can be carried on chromosome, plasmids, or conjugative transposons | Chromosomal ; can be transferred by conjugation |
| Confers cross-resistance to macrolides, lincosamides, and streptogramins | Does not affect 16-member macrolides (e.g., josamycin), lincosamides or streptogramins |
| High-grade resistance to erythromycin (MIC>64μg/ml) | Intermediate levels of resistance to erythromycin (MIC 1-32μg/ml) |
| Predominant mechanism in Europe and South Africa | Predominant mechanism in North America and Asia |

Ref. : Lynch III and Martinez, 2002.

### 2.3.3 Pneumococci Resistant to Sulfonamides

The sulfonamide agents inhibit activity of bacteria by competing with para-aminobenzoate binding to dihydropteroate syntase in folic pathway. Sulfonamide-resistant pneumococci have acquisition of a 6-bp insertion in *sul*A, a chromosomal gene encoding dihydropteroate syntase. Therefore, sulfonamide has low affinity to dihydropteroate syntase if pneumococci carry mutation *sul*A gene (Maskell, *et al*, 1997).

### 2.3.4 Pneumococci Resistant to Fluoroquinolones

At least two mechanisms appear to be necessary to the presence of fluoroquinolone-resistance. First, *par*C mutation leads to low-level resistance, this mutation is ready to undergo the second mutation, which involves DNA gyrase (*gyr*A) and results in high-level resistance. Moreover *pmr*A, a gene encoding an efflux pump is

also the important cause of decreased accumulation to these agents for pneumococci (Barry, 1999; Piddock, 1999). Recently, Piddock and colleagues (2002) proposed in their study that *par*C, *par*E and *gyr*A were the predominantly mutation genes of pneumococci for resistant to fluoroquinolones; in contrast, the expression of *pmr*A was not gratefully associated with a phenotype suggestive of an efflux mutation.

### 2.3.5 Pneumococci Resistant to Tetracyclines

Ribosomal protection mechanism is the main cause tetracycline resistance that is the result from the *tet*M gene which appears to be marker on transposon Tn 1545. However, some strains that possess the *tet*O gene have been associated with this resistance (Tomasz, 1997; Crook and Spratt, 1998).

## 2.4 History of Drug-Resistant *Streptococcus pneumoniae*

Community-acquired *Streptococcus pneumoniae* infection was a major caused of morbidity and mortality in the pre-penicillin era. After widespread using penicillin for pneumococcal infection, the fatality rate with pneumococcal pneumonia was reduced from 20% to 5%; with pneumococcal bacteremia from 50% to 20%; with pneumococcal meningitis from 80%-100% to 30% (Tomasz, 1997). However, penicillin-resistant pneumococci had not been reported until the mid-1960s in Papue New Guinea, Australia and South Africa. Besides penicillin resistance, other antimicrobial resistant and multidrug-resistant pnemococci became endemic in commonplace. Optochin, was one of the first drugs of pneumococcal infection therapy, had been reported of resistance in laboratory animal in 1912 before used in human. Five years later, optochin-resistant pneumococcal isolate was reported (Harwell and Brown, 2000). In addition, other drugs-resistant strains were first recognized as followed: sulfonamide in 1943; tetracycline in 1962; erythromycin in 1967 and chloramphenicol in 1970 (Barry, 1999). The next dramatic event in the epidemiology of drug-resistant pneumococci was the

outbreak of pneumococcal disease caused by multidrug-resistant strains in South Africa hospitals in epidemics in 1977 (Harwell and Brown, 2000).

## 2.5 Prevalence of Drug-Resistant *Streptococcus pneumoniae*

In the United States, the estimated annual number of pneumococcal infections are about 0.5 million cases of community-acquired pneumonia; 50,000 cases of bacteremia; 3,000-5,000 cases of meningitis, and more than 7 million cases of otitis media. Mortality rates of pneumococcal pneumonia or bacteremia were also ranging from 9% to 30%. The prevalence of drug-resistant *Streptococcus pneumoniae* is still rising, particularly in the past 5-10 years (Jacobs, 1999; Chenoweth, *et al*, 2000). A nation surveillance study conducted in 1999-2000 with 33 the Unites States medical centers reported that *S.pneumoniae* were overall penicillin-nonsusceptible at 34.2% (MIC $\geq$ 0.12 μg/ml) and 21.5% were high-level penicillin resistance (MIC $\geq$ 2 μg/ml). Resistance rates among other antibiotic agents were as the following: 25.2-25.7% for macrolides, 8.9% for clindamycin, 16.3% for tetracycline, 8.3% for chloramphenicol, 30.3% for co-trimoxazole and 22.4% for multiple drugs. These resistance rates were increased from 1994-1995 as the following: 10.6% for penicillin, 16.1% for erythromycin, 9.0% for tetracycline, 9.1% for co-trimoxazole, 4.0% for chloramphenicol, and 13.3% for multiple drugs. Despite awareness and prevention efforts, antimicrobial resistant with pneumococci continues to increase in the United States (Doern, *et al*, 2001). The TRUST IV (Tracking resistance in the United States today) surveillance program also analyzed susceptibility of *Streptococcus pneumoniae* from 239 laboratories that were distributed across 9 regions in the United States in 1999-2000 and compared with TRUST III study (1998-1999). The significant increasing in percentage of resistant isolates were 3.7% to amoxicillin-clavulanate ($P$<0.001); 2.2% to cefuroxime ($P$<0.05); 3.1% to clarithromycin ($P$<0.001); and 2% to co-trimoxazole ($P$<0.05). Penicillin-resistant isolates were increased by 1.3% ($P$=0.05). This study found that penicillin-nonsusceptible strains were 33.1%, which was similar to Doern *et al* study. However, high-level penicillin-resistant pneumococci being

reported in TRUST IV study (16.0%) was less than Doern *et al* study (21.5%) (Thornsberry, *et al*, 2002). Multidrug-resistant isolates were increased from 9% in 1995 to 14% in 1998 (Whitney, *et al*, 2000) and to 22.4% in 1999-2000 (Doern, *et al*, 2001). Even though, the numbers from those study were slightly more than Thornsberry and colleagues study who found that multidrug-resistant pneumococci were increased from 11.0% in 1998-1999 to 12.2% in 1999-2000. The investigators suggested that levofloxacin-resistant pneumococci remained rare (0.05%), and the figure was quite similar with only less than 1% from Whitney *et al* study (2000).

In Asia and Europe, a multicenter study was performed during the winter of 1997-1998. Penicillin-nonsusceptible pneumococci were determined from 48 sites in China, France, Germany, Italy, Japan, Spain, and UK as 16.9, 66.5, 7.8, 16.8, 54.1, 65.6, and 10.8, respectively (Sahm, *et al*, 2000). Moreover, the prevalence in Hungary showed that penicillin-susceptible strains, penicillin-intermediately susceptible strains and penicillin resistance were 69.1%, 21.7% and 9.2%, respectively. Cefotaxime-resistant pneumococci had emerged by 20% of all 96 penicillin-nonsusceptible strains. The four high-level levofloxacin resistant strains (MIC$\geq$32 μg/ml) were found (Glatz, *et al*, 2001). In addition, Granizo *et al* (2000) did a study in Spain and found that erythromycin resistance correlated with taking macrolide twice daily, and prevalence of high-level penicillin-resistance also related with the increase in the consumption of oral cephalosporins. Moreover, they found that the prevalence increasing of high-level penicillin and erythromycin resistance was strongly correlated with each other. Another studies in Asia, the Asian Network for Surveillance of Resistant Pathogens (ANSORP) carried out a multicenter in vitro surveillance study of pneumococcal drug resistance in 11 Asian countries in 1996-1997. Korea had the greatest frequency of non-susceptible strains to penicillin with 79.7%, followed by Japan (65.3%), Vietnam (60.8%), Thailand (57.9%), Sri-Lanka (41.2%), Taiwan (38.7%), Singapore (23.1%), Indonesia (21.0%), China (9.8%), Malaysia (9.0%), and India (3.8%) (Song, *et al*, 1999). Prevalence of penicillin-nonsusceptible pneumococci was increased by 76% in Taiwan during 1998-1999. In the same study, high rate of non-susceptible isolates to extended-

cephalosporin (56%), azithromycin (94%), clarithromycin (95%), and co-trimoxazole (65%) were also reported (Hsueh, *et al*, 2000). In addition, macrolide-resistant pneumococci were studied in Hong Kong. The results were shown that erythromycin resistance was 38% among penicillin-susceptible strains and 92% among penicillin-nonsusceptible pneumococci (Margaret, *et al*, 2001). Furthermore, in New Zealand, 113 of 216 cefotaxime-resistant isolates were high-level cefotaxime resistance (MIC $\geq$ 4 μg/ml), and almost of 113 isolates were serotype 19F (Brett, 2001).

In Thailand, there were many investigators studied about surveillance of drug-nonsusceptible *Streptococcus pneumoniae.* Firstly, Bamrungtrakul and colleagues (1994) found that 8.7 % of pneumococcal isolates resisted to penicillin, 25.4% resisted to co-trimoxazole, and no chloramphenicol resistance was found. Secondly, Pancharoen and colleagues (2001) studied in Thai children with systemic pneumococcal infection between 1986-1997. The percentage of susceptible isolates to penicillin, chloramphenicol, cefotaxime or ceftriaxone, ciprofloxacin, imipenem, and vancomycin were found as 69.6, 91.3, 100, 87.2, 100, 97.1%, respectively. Thirdly, penicillin-nonsusceptible strains were found 10.6% at Siriraj Hospital, a university hospital in Bangkok, in 1986-1987 (Komolpis, *et al*, 1991). The figures had increased to 30% in 1995, to 67.6% in 1996, but dropped to 58.8% in 1997. However, high-level penicillin resistance increased rapidly from none in 1995 to 11% in 1996, and to 23% in 1997 (Aswapokee, *et al*, 1998). After that, the percentage of high-level penicillin resistance was decreased to 21.1% in 1998 (Chokephaibulkit, *et al*, 2000). Finally, the susceptibility of pneumococci was found, particularly at Hat Yai Hospital in the south of Thailand, high-level penicillin resistance were increased from 1.2% in 1993-1994 to 5% in 2000-2001, and penicillin-nonsusceptible strains were increased from 16.4% in 1993-1994 to 43% in 2000-2001 (Chup-uppakarn, 1998; Warachit, *et al*, 2002). It may accept that emergence of drug-resistant pneumococci is rising worldwide, including multidrug-resistance.

## 2.6 Risk Factors for Infected Drug-Resistant *Streptococcus pneumoniae*

The emergence of antimicrobial resistance is due to excessive and often unnecessary and/or inappropriate use of antibiotics in human. The increasing of antibiotics use in animal farms also affects the antimicrobial resistance. Spread of drug-resistant pneumococci occurs in the community firstly, resulting from overcrowding and using antibiotics to treat viral respiratory illness (Rao, 1998). On the other hand, risk factors of drug-resistant pneumococcal infections in children include prior antibiotic use, age of less than 2 years, residence in day care or nursing home, recent hospitalization, and underlying immunosuppressive disease. Among adults, recent studies found a higher rate of antibiotic-resistant pneumococci from HIV-infection patients. Alcoholism and age of more than 65 years also were associated with an increasing risk. Some of the same risk factors for children also were found in adults, such as recent antibiotic use and hospitalization (Bonafede and Rice, 1997; Chenoweth, *et al*, 2000; Harwell and Brown, 2000). Deeks and colleagues (1999) studied in hospitalized children with pneumococci infection in Uruguay and Argentina. They found that risk factors of drug-resistant pneumococci infection were penicillin or ampicillin usage in the 3 months before illness (OR=2.9; 95%CI 1.5-5.7) and possession of private medical coverage (OR=2.4; 95%CI 1.2-5.0). In Thailand, Chokephaibulkit *et al* (2000) found that risk factor associated with penicillin-resistant pneumococcal infection was antibiotic usage within 3 weeks that was similar to Dejthevaporn and colleagues study (2000). Another study was the case-control study of levofloxacin-resistant pneumococci in Hong Kong. Risk factors of infected with levofloxacin-resistant strains were older age, residence in a nursing home, history of recent and multiple hospitlaization, recent exposure of fluoroquinolone and beta-lactam antibioltics, presentation of chronic disease, and nosocomial origin of bacteria (Ho, *et al*, 2001).

## 2.7 Clinical Outcome of Infection Caused by Drug-resistant *Streptococcus pneumoniae*

### 2.7.1 Pneumococcal Pneumonia and Bacteremia

In adults, Pallares and colleagues (1995) proposed in their 10-year prospective study of the effect of resistance to penicillin and cephalosporin in 504 adults that elevating of mortality rate was not correlated with levels of resistance to penicillin and cephalosporin. The mortality was 25% of 24 patients with penicillin-nonsusceptible strains who were treated with penicillin or ampicillin, while 19% of 129 patients with penicillin were infected with penicillin-susceptible strains ($P$=0.51). Among patients who were treated with cefotaxime or ceftriaxone, mortality was 22% in 59 with penicillin-nonsusceptible strains and 25% in 127 with penicillin-susceptible strains ($P$=0.64). In the same way, Feikin *et al* (2000) concluded that mortality was not increased in most patients with infection of β-lactam resistant pneumococci, even though older age and underlying disease were the most important factors influencing death from pneumococcal pneumonia. However, this study found that the risk of death after the fourth admission day was associated with highly penicillin resistance (MIC 4 μg/ml). Another study compared clinical presentations and clinical outcomes of pneumonia caused by antimicrobial resistance versus those with caused by antimicrobial-nonsusceptible strains. It revealed that mortality or requirement of specific care in intensive care did not significantly differ in both groups (Moroney, *et al*, 2001). In contrast, Metlay and team (2000) found that patients whose insolates were non-susceptible to penicillin had statistically significant greatly risk of in-hospital death due to pneumonia (RR 2.1; 95%CI 1-4.3), and supportive complications of infection (RR 4.5; 95%CI 1-19.3). However, after adjustment of baseline difference in severity of illness, only risk of supportive complication was statistically significant. In addition, they concluded that increasing risk of adverse outcome associated with penicillin-nonsusceptible pneumococci.

In children, Friedland (1995) studied in 108 children with pneumococcal infections. In children with pneumonia, 88% of penicillin-nonsusceptible infections treated with ampicillin or other equivalent β-lactam agents showed clinical improvement by 7 days of therapy compared with 93% with penicillin-susceptible pneumococcal infections (OR, 1.9; 95% CI, 0.3-15.9). Concordant with many studies of pneumococcal pneumonia, there were no statistically significant of the clinical presentation and outcomes of therapy between patients with penicillin-susceptible and-nonsusceptible pneumococci. But, hospitalization was the most closely related to underlying disease and clinical status at presentation (Choi and Lee, 1998; Tan, *et al*, 1998; Kaplan, *et al*, 2001). They recommended that standard β-lactam antibiotic therapies were still highly effective, though, intermediate-penicillin resistance was a little of clinical significant in pneumococcal pneumonia (Silverstein, *et al*, 1999).

### 2.7.2 Pneumococcal Meningitis

Pneumococcal meningitis, the rare disease and life threatening caused extremely high mortality. Pneumococcal isolates were the major pathogen for bacterial meningitis and they were reported as the highest fatality causes in the United States in the past 5 years. The rate of pneumococcal meningitis was 1.1 per 100,000 persons in the United States in 1995. Pneumococci were the predominant pathogen both in newborn and adults. Mortality among adults with pneumococcal meningitis (21%) was higher than those with pneumococcal pneumonia (13%) (Schuchat, *et al*, 1997). Stanek and Mufson (1999) studied in 20-year surveillance of pneumococcal meningitis from 1978-1977. They found that 30.9% of patients were children and 8.3% of them were died. The mortality rate in adults (69.1%) was higher than in children (36.8%) that was relevant with Fiore and colleagues study (2000).

Unfortunately, there was a case report of cefotaxime treatment failure in an adult with meningitis who was infected by cefotaxime-resistant pneumococci (MIC = 2 μg/ml)

(Pacheco, *et al*, 1997). Moreover, Choi and Lee (1998) reported that 7of 11-meningitis illness children were due to penicillin-resistant isolates. They had unfavorable response notes with ampicillin and cholramphenicol treatment and were subsequently treated with vancomycin. Other 2 patients with initiate treated with cefotaxime also subsequently treated with vancomycin since treatment failure. In addition to other studies of pneumococcal meningitis among children, Olivier *et al* (2000) studied the outcome of children with cefotaxime- or ceftriaxone-susceptible and –nonsusceptible *Streptococcus pneumoniae* meningitis. Bacteriologic failure had been reported in patients with meningitis caused by strains with decreased susceptibility to cefotaxime or ceftriaxone. Initial antibiotic regimens were prescribed with antimicrobial combination (mostly cefotaxime or ceftriaxone combined with vancomycin), as recommended. These results were similar to Fiore *et al* (2000), studying in 109 children. They concluded that mortality, length of hospital or ICU stay, frequency of neurological sequelae and need for supplemental oxygen, mechanical ventilation, ICU care or extended-care-facility admission were similar among patients with cefotaxime-susceptible and -nonsusceptible pneumococcal meningitis.

### 2.7.3 Otitis Media

In the United States, in 1998, otitis media in children was reported more than 20 million cases, and 80% of them had at least one episode of otitis media. Pneumococci were the most common cause of otitis media in childhood. Several episodes of otitis media and antibiotic prescribing to all children who have acute otitis media leaded to the selection pressure of resistant pneumococci easily. In 1997, a report studied in the United States revealed that highly penicillin-resistant pneumoccocci was 49.7% of 290 isolates collected from the middle ear of children. Unfortunately, difficulty of drug accumulation in middle ear fluid remains problem. Hence, increasing rate of treatment failure in otitis media associated with selected administered cephalosporin and macrolides was reported (Dagan, *et al*, 1996,1997).

## 2.8 Management of Pneumococcal Infectious Diseases

### 2.8.1 Pneumonia

For community-acquired pneumonia in outpatients, the new guidelines in both of North America, the American Thoracic Society and the Infectious Disease Society of America, recommended macrolides, doxycycline, or an antipneumococcal fluoroquinolone (e.g., levofloxacin, moxifloxacin, gatifloxacin) for empirical therapy. The rationale was to atypical pathogen coverage (*Mycoplasma pneumoniae* and *Chlamydia pneumoniae*). Amoxicillin was also the recommendation for the treatment as appropriate empirical therapy (Jacobs, 1999). Antipneumococcal fluoroquinolone may be preferred choice for older patients or those with underlying disease. However, some guideline stated that because of reporting of levofloxacin-resistant pneumococci (Musher, 2000; Davidson, *et al*, 2002), fluoroquinolone should be reserved for cases infected with drug-resistant pneumococci or those cases associated with treatment failure or allergy to other antibiotics (Harwell and Brown, 2000; File Jr., 2002). In Europe, the British Thoracic Society, recommended amoxicillin (500 mg to 1 g, three times daily) or erythromycin (500 mg, four times daily), or clarithromycin (500 mg twice daily). Tetracycline was not recommended since its safety. The duration of therapy was recommended for 7 days for non-severe pneumonia, and 14-21 days for severe infections or those caused by atypical pathogens (British Thoracic Society, 2001; Keeley, 2002). The ketolides may become to optimal therapy for outpatient resulting to oral administration available.

For the more seriously illness, hospitalized patients, the antibiotic of choice was parenteral penicillin G for infections due to susceptible strains. Optimal therapy for severe pneumococcal pneumonia due to resistant strains and also intermediate susceptible strains were controversial. Interestingly, favorable responses had been noted with high dose penicillin G for pneumococcal pneumonia caused by intermediately susceptible to penicillin and resistant strains, resembling with cefotaxime

and ceftriaxone. Thus, many studies and guidelines recommended that hospitalized patients with pneumococcal pneumonia caused by susceptible or intermediately susceptible to penicillin responsed to treatment with parenteral penicillin (2 MU q4h), ampicillin (1 g q6h), ceftiaxone (1 g q24h), or cefotaxime (1 g q8h). On the other hand, pneumonia caused by penicillin- or cefphalosporin-resistant strains should be treated with high dose of these drugs. The newer fluoroquinolones and vancomycin may have a role for infections due to isolates with penicillin or cephalosporin MIC of more than 4 μg/ml. Other agents that recent available are carbapenems (imipenem and meropenem), quinupristin-dalfopristin and linezolid. However, determining the antibiotics use should be concern about local susceptibility pattern, antibiotic selection of resistant pneumococci, and economic impact (Table 3 and Table 4) (Jacobs, 1999; Klugman and Feldman, 1999; Chenoweth, *et al*, 2000; Harwell and Brown, 2000; Musher, 2000; File Jr., 2002).

**Table 3** β-lactam antibiotics for treatment of drug-resistant pneumococcal pneumonia for adults.

| Drugs | Total daily dosage | Route/Inverval |
|---|---|---|
| Penicillin G | ≥ 12 MU/d | IV q4h to q6h or continuous |
| Ampicillin | 8-12 g/d | IV q4h to q 6 h |
| Amoxicillin | 2-3 g/d | po q8h to q12h |
| Ticarcillin | 12-18 g/d | IV q4h to q6h |
| Piperacillin | 12-16 g/d | IV q6h |
| Cefprozil | 1 g/d | po q12h |
| Cefaclor | 3 g/d | po q8h |
| Cephalexin | 3-4 g/d | po q6h to q 8h |
| Cefuroxime | 6-9 g/d | IV q8h |
| Cefuroxime | 1-2 g/d | po q8h to q 12h |
| Cefpodoxime | 800 mg/d | po q12h |
| Ceftriaxone | 2-4 g/d | IV q12h to q24h |
| Cefotaxime | 2-4 g/d | IV q6h to q8h |
| Cefepime | 2-6 g/d | IV q8h to q12h |
| Imipenem | 2-3 g/d | IV q6h to q8h |
| Meropenem | 3 g/d | IV q8h |

Ref. : Harwell and Brown, 2000

Table 4 Non β-lactam antibiotics for treatment of drug-resistant pneumococcal pneumonia for adults.

| Drugs | Total daily dosage | Route/Inverval |
|---|---|---|
| Vancomycin | 2-3 g/d | IV q6h to q12h |
| Rifampin | 600 mg/d | po/IV q12h to q24h |
| Levofloxacin | 500 mg/d | po/IV q24h |
| Sparfloxacin | 400 mg/d | po q24h |
| Grepafloxacin | 600 mg/d | po q24h |
| Trovafloxacin | 100-200 mg/d | po q24d |
| Alatrofloxacin | 200-300 mg/d | IV q24h |
| Gatifloxacin | 400 mg/d | po/IV g24h |
| Moxifloxacin | 400 mg/d | po q24h |
| Doxycycline | 200 mg/d | po/IV q12 to q24h |
| Chloramphenicol | 50-100 mg/kg/d | po/IV q6h |
| Co-trimoxazole | 320-1,600 mg/d | po/IV q12h |
| Erythromycin | 30-50 mg/kg/d | po/IV q6h to q8h |
| Azithromycin | 500, then 250 mg/d | po/IV q12h |
| Clarithromycin | 1,000 mg/d | po q12h |
| Clindamycin | 900-1,800 mg/d | po/IV q8h |
| Linezolid | 1,200 mg/d | po/IV q12h |

Ref : Harwell and Brown, 2000.

For children with pneumococcal pneumonia, although case reports of treatment failures using standard β-lactam therapy have been established, the publication was still rare. Standard antibiotics remained effective for children with pneumococcal infections. Most guidelines suggested initial treatment with oral amoxicillin or penicillin or parenteral cefuroxime when patients required hospitalization. For outpatients, oral amoxicillin was effective drug for infection with penicillin-susceptible pneumococci. If pencillin-resistance was prevalent in a community, intravenous β-lactam antibiotic or macrolides

or both drugs was usually indicated. The Drug-Resistant *Streptococcus pneumoniae* Therapeutic Working Group (DRSPTWG), in May 2000, identified oral β-lactam, including cefuroxime axetil, amoxicillin and amoxicillin/clavulanate as drug of choice for ambulatory community-acquired pneumonia. On the other hand, macrolides were not considered appropriate for children younger than 5 years because this young age group was not necessary to use broad-spectrum antibiotic for coverage atypical pathogen. For immunocompromised patients, they often exposed antibiotics for therapy or prophylaxis that may be increase risk of drug-nonsusceptible pneumococcal infection. Some experts would initiate empiric treatment with vancomycin and cefotaxime or ceftriaxone untill susceptibility results were available. Later therapy should be based on antibiotics susceptible results and clinical of patients (American Academy of Pediatrics, 1997; McCracken JR, 2000).

### 2.8.2 Meningitis

Penicillin is ineffective against drug-nonsusceptible pneumococci because levels in CSF were reached only 2-10% of serum levels. Cefotaxime or ceftriaxone was drug of choice for empiric therapy for meningitis with suspected pneumococci because of the capability of achieving reliable concentrations in the CSF although infection caused by penicillin-nonsusceptible strains. Some physicians suggested that vancomycin was the optimal for initiate empiric therapy because of its more certain antimicrobial efficacy, it crossed the blood brain barrier more reliably, and the organism may be susceptible. Starting dosages of vancomycin should be used high doses initially and then adjusted downward to maintain a serum level of 40 to 50 μg/ml (60-80 mg/kg/d q6h in children and 2.5-3 g/d q12h in adults). Another alternative drug is meropenem since broad-spectrum activity and with a few resistant strains identified. There were many studies added rifampin to ceftriaxone or vancomycin and claimed a better outcome in the treatment of pneumococcal meningitis. On the other hand, a systemic study showed indifference or antagonism when rifampin was added to β-lactam antibiotics. Currently,

Cottagnoud and colleagues (2002) did try to treat pneumococcal meningitis with gentamicin in an animal model. They found that gentamicin monotherapy had less efficacy than vancomycin monotherapy, whereas combination of vancomycin with gentamicin was significantly superior compared to either monotherapy alone. That may be the alternative way to treat pneumococcal meningitis in the future. Combination therapy with steroids should be caution when vancomycin was mainly therapy due to reducing permissive inflammation and limiting drug penetration of vancomycin. In addition, the effectiveness of dexamethasone for preventing sequelae of pneumococcal meningitis for children was unproved; however, there were a few studies showed that dexamethasone therapy can decrease fever and prevent hearing loss or other sequelae in children with pneumococcal meningitis. Dosage and regimens of antimicrobials for children with pneumococcal meningitis therapy were showed in Table 5 (American Academy of Pediatrics, 1997; Jacobs, 1999; Klugman and Feldman, 1999; Chenoweth, *et al*, 2000; Harwell and Brown, 2000; Musher, 2000; File Jr., 2002).

### 2.8.3 Otitis Media

The recommendation therapy for pneumococcal otitis media in children remained amoxicillin, based on this agent had likely better response than other drugs to treat penicillin-susceptible and -intermediately susceptible pneumococci. Moreover, it was possible to achieve middle ear fluid drug concentrations that exceed the MIC of most resistant isolates by administering 60-80 mg/kg/d twice daily. Another recommendation suggested higher doses 80-90 mg/kg/d of amoxicillin to manage resistant organism. Amoxicillin-clavulanate may be use in treatment failure cases for gram-negative coverage. Besides amoxicillin, alternative therapy for otitis media is oral cephalosporins; cefuroxime, cefixime, cefdinir. However, none of oral cephalosporins were more active than amoxicillin against either penicillin-susceptible or –resistant isolates. In view of macrolide resistance becomeed increasingly, macrolides were less valuable alternate antibiotics for otitis media (Klugman and Feldman, 1999; Harwell and Brown, 2000; Musher, 2000; Giebink, 2001).

**Table 5** Doses of intravenous antimicrobials for children with pneumococcal infections.

| Drugs | Meningitis | | Nonmeningeal Infections | |
|---|---|---|---|---|
| | Dose, kg/d | Dose Interval | Dose, kg/d | Dose Interval |
| Penicillin G | 250,000-400,000 U | q4h to q6h | 250,000-400,000 U | q4h to q6h |
| Cefotaxime | 225-300 mg | q6h to q8h | 150-225 mg | q6h to q8h |
| Ceftriaxone | 100 mg | q12h to q24h | 80-100 mg | q12h to q24h |
| Vancomycin | 6o mg | q6h | 40-60 mg | q6h |
| Rifampin | 20 mg | q12g | Not indicated | - |
| Chloramphenicol | 75-100 mg | q6h | 75-100 mg | q6h |
| Clindamycin | Not indicated | - | 25-40 mg | q6h to q8h |
| Imipenem+cilastatin | Not indicated | - | 60 mg | q6h |
| Meropenem | 120 mg | q8h | 60 mg | q8h |

Ref. : American Academy of Pediatrics, 1997

### 2.8.4 Prevention

Vaccination is the principle of preventive pneumococcal infections except limiting the selective pressure of antibiotic use and infection control. The modern pneumococcal vaccine was introduced in 1977 and expanded from the valent of 14 to 23 in 1983. Consideration is now being given to the routine use of effective pneumococcal conjugated vaccines in children and adults. More than 80% of vaccine efficacy had been showed, depending on age and immune competence of the host. Vaccine recommendation was listed in Table 6 for adults and Table 7 for children (Harwell and Brown, 2000; Musher, 2000).

## 2.9 Susceptibility Testing

Because of the *Streptococcus pneumoniae* is a gram positive fastidious organism, susceptibility testing should be interpreted with care. Many methods were used in the routine clinical laboratory to test the activity of antimicrobials against this pathogens. There were pneumococcal comprise agar dilution, broth microdilutions, E test, and disk diffusion. The simplest method for determine MIC is the E test, consisting of a continuous stable gradient of antimicrobial agent corresponding to 15 two-fold dilutions on a strip. Skulnick *et al* (1995) evaluated of accuracy and reproducibility of E test for pneumococci to penicillin and ceftriaxone. The results were good agreement between technologists for interpretation of the E test. Moreover, there were many studies compared other susceptibility testing methods with E test. They found an excellent correlation between the results obtained by disk diffusion, agar dilution, microdilution, and E test (Clark, *et al*, 1998; Wang, *et al*, 1998; Kelly, Jacobs and Appelbaum, 1999; Davies, *et al*, 2000). On the basis, it can deduce that E test has gained wide acceptance as an accurate method and has been current use in many studies.

Table 6 Pneumococcal vaccination for adults

| Condition | Timing |
|---|---|
| All persons age > 65 yrs. | Repeat in 5 yrs. |
| Chronic lung disease (COPD, cystic fibrosis) | Repeat in 5 yrs. |
| Heart disease | Repeat in 5 yrs. |
| Diabetes mellitus | Repeat in 5 yrs. |
| Nephrotic syndrome or renal failure | Repeat in 5 yrs. |
| Liver disease | Repeat in 5 yrs. |
| Splenectomy (functional or anatomic) | 2 wks before, if possible; Repeat in 5 yrs. |
| Organ transplantation | 2 wk before, if possible; Repeat in 5 yr |
| Immunosuppressive chemotherapy | 2 wks before, if possible; Repeat in 5 yrs. |
| HIV infection | Repeat in 5 yrs. |
| Recurrent pneumococcal infections | Repeat in 5 yrs. |

Ref. : Harwell and Brown, 2000.

Table 7 Pneumococcal vaccination for children ≤ 2 year

| Condition | Timming |
|---|---|
| Sickle cell disease | Repeat in 5 yrs. |
| Functional or anatomic asplenia | 2 wks before, if possible; Repeat in 5 yrs. |
| Nephrotic syndrome or chronic renal failure | Repeat in 5 yrs. |
| Immunosuppressive chemotherapy or transplantation | 2 wks before, if possible; Repeat in 5 yrs. |
| HIV infection | Repeat in 5 yrs. |
| CSF leaks | Repeat in 5 yrs. |
| Heart disease | Repeat in 5 yrs. |
| Chronic lung disease | Repeat in 5 yrs. |
| Chronic liver disease | Repeat in 5 yrs. |
| Living in high-risk environments and communities | Repeat in 5 yrs. |
| Recurrent pneumococcal infections | Repeat in 5 yrs. |

Ref. : Harwell and Brown, 2000.