Pneumocystis Pneumonia: An Update

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Pneumocystis pneumonia is a major cause of illness and death in immunocompromised hosts. The numbers of pneumocystis pneumonia cases in Thailand have increased each year from 1992 to 2000 and peaked in 2000 at 6,255 cases. The microbe that causes pneumocystis pneumonia in humans is called Pneumocystis jirovecii. Pneumocystis sp. was discovered nearly a century ago, but the knowledge of Pneumocystis sp. remained poorly understood, until the molecular biology techniques help scientists verify it's fungus nature. In the past, Pneumocystis sp. was misclassified as protozoan due to its morphologic features. Later, it was reclassified as fungus due to DNA analysis. Cotrimaxazole, the combination of trimethoprim-sulfamethoxazole, is the drug of choice for treatment and prophylaxis of pneumocystis pneumonia. However, increasing evidence of mutations in the enzyme dihydropteroate synthase (DHPS), the target of sulfa drugs represent emergence of sulfa resistance.

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Pneumocystis sp. is an atypical fungus that remains a serious cause of sickness and death in immunocompromised patients. The *Pneumocystis* organism was first identified as a protozoan nearly 100 years ago by Carlos Chagas [1]. Based on advanced molecular studies, *Pneumocystis* has been reclassified as a fungus by using DNA sequence analysis of srDNA genes [2,3]. *Pneumocystis* infection is host species specific [4]. Human specific *Pneumocystis* has been recently renamed *Pneumocystis jirovecii* [5]. The prevalence of pneumocystis pneumonia has increased in AIDS patients especially in those who do not receive adequate antiretroviral drug. Trimethoprim-sulfamethoxazole remains the first-line drug for treatment and prophylaxis of pneumocystis pneumonia. However, accumulating evidence has demonstrated that the gene mutations in enzyme dihydropteroate synthase (DPHS), the target of sulfa drugs, appear to represent emerging resistance in pneumocystis pneumonia [6].

Epidemiology of Pneumocystis organism

Historically, *Pneumocysti carinii* was first identified in the early 1900s in trypanosome-infected lungs of animals by Carlos Chagas, who believed it was a form of trypanosome [1]. Subsequently, Antoni Carinii identified the same organism in infected rat lung [7]. Several years later, Delanoes recognized that Chagas and Carinii had identified a new genus and it was named *Pneumocystis carinii* in honor of Carinii [8]. In the 1930s and 1940s, pneumocystis pneumonia was associated with premature and malnourished infants in Europe and subsequently with patients undergoing organ transplantation, or receiving chemotherapy for the treatment of malignant disease [9]. Recently, the highest incidence of pneumocystis pneumonia is in AIDS patients and the number of recognized cases has increased since 1993.

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Cases of Pneumocystis Pneumonia in Thailand

Before 1992, there were fewer than 100 cases per year of pneumocystis pneumonia in Thailand. After 1993, there was a marked increase in the incidence of cases reported to the Thai Ministry of Public Health that peaked in 2000 at 6,255 cases per year (Fig. 1)[10]. In most developed countries, pneumocystis pneumonia has been the most common AIDS-defining infections since the beginning of the epidemic, accounting for nearly 67% of all initial AIDS diagnosis[11]. However, in Thailand, pneumocystis pneumonia was reported in 19.8% of patients with AIDS because many of these diagnoses were made on clinical grounds alone, these data may not accurately reflect the true incidence of pneumocystis pneumonia[12]. In 2002, there was a minimal decline in the number of pneumocystis pneumonia cases that may be due to the use of pneumocystis pneumonia prophylaxis and antiretroviral therapy. In

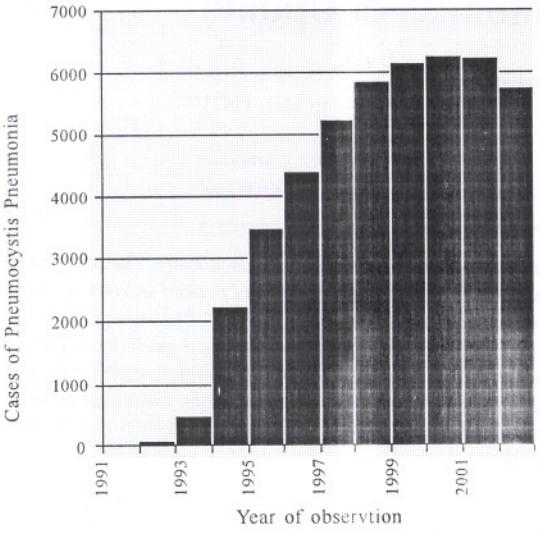

Fig. 1 Cases of pneumocystis pneumonia reported to the Thai Ministry of Public Health, 1991-2002

developing countries, primary prophylaxis for opportunistic infections such as pneumocystis pneumonia, toxoplasmosis, cytomegalovirus (CMV) infection and Mycobacterium Avium Complex (MAC) infection are recommended and considered as a standard of care [13]. However, these approaches are compromised by limited resources and should be implemented differently in each country. In Thailand, the treatment and prevention of opportunistic infections (OI) are strongly advocated by Thai policy because it is considered to be cheaper and more affordable than antiretroviral drugs due to the high cost [14]. Primary prophylaxis of pneumocystis pneumonia, using a combination of trimethoprim-sulfamethoxazole as a first-line drug, is recommended as standard care, because it is the most cost-effective and widely implemented regimen for HIV cases in Thailand [13].

Advances in understanding the biology of Pneumocystis organisms

The knowledge on biology of *Pneumocystis sp.* has been investigated for many decades. *Pneumocystis* organisms were first identified as a protozoan because of the morphologic features [8]. Based on advanced molecular studies, *Pneumocystis sp.* has been reclassified as a fungus by analysis of the srRNA subunit [15,16]. *Pneumocystis* sp. was originally thought to be only one strain that was capable of infecting many different mammalian host species. However, advanced studies have shown that there are many different types of *Pneumocystis* organisms, each of which is restricted to infecting a single host species [17-19].

Pneumocystis sp. that infects humans, which is *P. carinii f. sp. hominis*, cannot infect mice, rats, or even monkeys. This organism was recently renamed *P. jirovecii*, in honor of the Czech parasitologist Otto Jirovecii, according to the requirements of the International Code of Botanical Nomenclature (ICBN) [20,21]. *Pneumocystis carinii* was retained for one of the two *Pneumocystis* species inhabiting rats [20,21]. Therefore, *Pneumocystis carinii* pneumonia (PCP) is referred to pneumocystis pneumonia because of taking the species name out of the disease name. *P. carinii* genome project has identified about 4,000 genes in rat *P. carinii*. The sequencing of cDNA expression has revealed about 2,000 genes that share homology with known sequences 1,412 of which were fungi (Table 1). *Pneumocystis* is now classified as an Archiascomycetous fungus [22,23].

Life Cycle and Morphology

The life cycle of *Pneumocystis* is still not clearly understood because these organisms cannot be cultured. This organism was studied on microscopic observation in mammalian lungs and in vitro culture. The various stages of the organisms include ascospores, pre-asci, asci and trophic forms are seen in alveolar spaces in host lung cells.

The *Pneumocystis* life cycle are seen as starting with the release of ascospores from the ascus (Fig. 2). These ascospores are haploid and smaller than mature trophic forms. They have an amoeboid form, several small knob-like projections extending outward, which were misunderstood to be a protozoan. The released scoopers conjugate in pairs to form the tropic forms. The electron microscope demonstrates that the

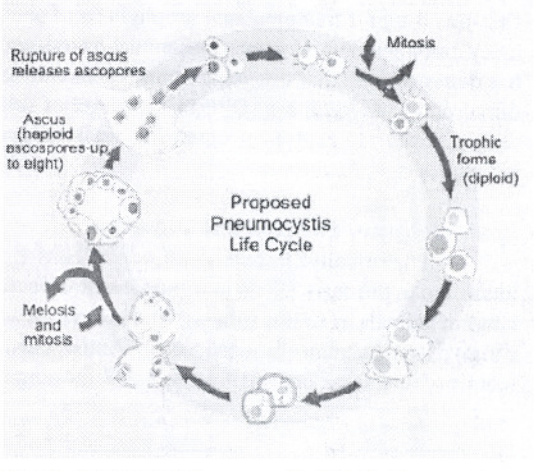

Fig. 2 Proposed of *Pneumocystis* Life Cycle

Table 1. Distinguish *Pneumocystis sp.* between fungi and protozoan features

Protozoan features	*Fungus features*
1. Strong similarities in microbe morphology and host pathology	1. The sequencing of cDNA expression about 2,000 genes, which sharing homology with known sequences 1,412 of which were fungi
2. Absence of some phenotypic features typical of fungi	2. Lacking in esgosterol
3. Presence of morphologic features typical of protozoan	3. Absence of structure for motibity
4. Ineffectiveness of antifungal drugs	4. Absence of structure for phagocytic
5. Effectiveness of drugs generally used to treat protozoa	5. Very difficult to culture.
6. Life cycle similarities to protozoan	6. Similarities in fungi cell wall

nucleus of the tropic is much larger than that of the ascosporic forms because it is now diploid. It supports the hypothesis of conjugation of encysted organisms [24]. At this stage, an organelle conducts the alignment of homologous chromosomes, which suggesting the presence of meiosis replication [25]. Mitotic divisions was followed after meiosis division in late pre-asci resulting in the formation ultimately of eight nuclei. The body of the ascus produces the nucleated ascospores.

Pneumocystis organisms adhere to each other and to type 1 pneumocytes, but not to type 2 pneumocytes [26]. The adherence mechanism is still unknown. *P. carinii* is found tightly adhere with alveolar and adjacent trophozoite cell membrane [27]. Numerous glycoproteins, which function as adhesions, have been identified on its surface [28,29]. One glycoprotein, gp120, has been demonstrated to bind fibronectin and may function in the formation of a fibronectin bridge between *P. carinii* and host epithelium [30]. However, the significance of this attachment in pathogenesis is still not fully understood.

The cyst of *P. carinii* is a spherical to ovoid structure 4 to 6 m in diameter. It has a three-layered cell wall and usually contains up to eight pleomorphic sporozoites (Fig. 3, 4). The trophozoite are 1-5 m long, uninucleated, ameboid structures with a a double-layered wall. Precysts are approximately 5 m. long, oval, smooth, and have a thick cell wall [31]. The *Pneumocystis* organisms are seen as foamy intra-alveolar exudates the nucleus of which has faint basophilic dots. The methenamine silver stain can stain the asci only, which comprises about 10-30% of the organisms. The Diff-Quik (Hemacolor) stain visualized all structures of the organism except the ascus wall. A research suggests Diff-Quik is superior to the silver stain because the organism can be identified easier [32].

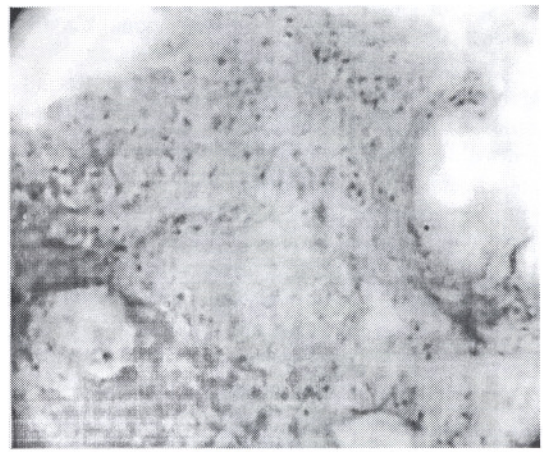

Fig. 3 Giemsa stain of the fluid sputum materials, observed at 1000 x magnification, shows cluster of *Pneumocystis* trophozoites and intracystis bodies of *Pneumocystis*, although the cyst walls do not stain

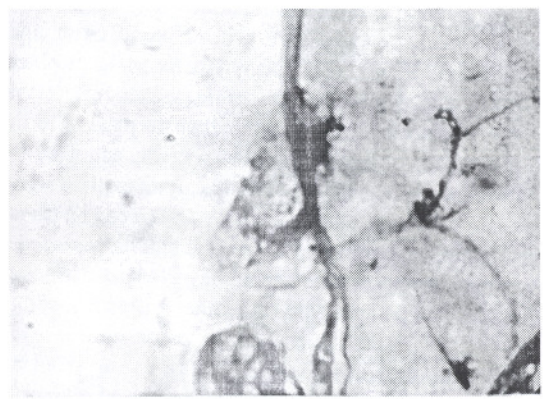

Fig. 4 A mature cyst containing 8 intracystic bodies is seen in Giemsa stained smear, observed at 1000 x magnification

Transmission of Pneumocystis sp

Mode of transmission in humans is unknown, though an airborne route is likely important through a series of animal experiments. This study demonstrated transmission from infected rats to susceptible immunocompromised rats in close contact [33]. For a decade, it

was thought that pneumocystis pneumonia resulted from reactivation of latent infection [34-37]. The organism remains latent within the host, subsequently, if the host's immune system fails, the latent *P. carinii* can reactivate and cause disease. A research found that healthy children with no detectable anti-antibodies at birth or in the first 3 months of life began to demonstrate a titer at 7 months of age [33]. In the present study, 83% of healthy children had at least 1:16 by the age of 4 years. This studies may support the hypothesis that *P. carinii* can exist in its host for long periods of time with asymptomatic of pneumocystis pneumonia. Several years later, there was accumulating evidence against the theory of reactivation of latent infection. Recent studies using sensitive and specific molecular techniques did not find *P. cavinii* in healthy immunocompetent hosts [38,39]. A experiment did not find *P. cavinii* in immunocompetent hosts after treatment of pneumocystis pneumonia and the presence of genotype switching in repeat episodes of pneumocystis pneumonia, and geographic variability in the disease which did not support the reactivation of latent infection theory [40]. Several studies have reported that different *P. carinii genotype* are present during repeat episodes of *P. carinii* in the same patient [41,42]. It cannot explain following reactivation of the latent theory because if this theory is true the genotype of *P. carinii* should be constant. Another argument against the latency hypothesis from studies of the geographic distribution of *Pneumocystis* strains and infection with *Pneumocystis*. Several studies demonstrated that frequencies of *P. carinii* genotypes vary in different cities and countries [43,44] and the strains reflect the patient's place of infection. Moreover, the strains better reflect the patient's place of infection rather than the place of birth, implying that infection has been recently acquired [44]. Subsequently, the latency theory cannot explain some situations. The possibility that *P. carinii* can be transmitted from person to person. The studies reported a group of pneumocystis pneumonia patients, with malignancies had contact with each other within a hospital. It was suggested that person to person transmission of pneumocystis pneumonia may occur from an infected susceptible immunocompromised patient in close contact [45,46]. Recently, evidence is the most likely mode of acquiring new infection from person to person. However, significant evidence of transmission of pneumocystis pneumonia does not have at this period.

Clinical Presentation of Pneumocystis Pneumonia

Pneumocystis sp. produces disease when alveoli become diffusely packed with organisms, which these organisms are usually accompanied by inflammatory cell interstitial reaction. Pneumocystis pneumonia results in a lung that is stiffened, which loading breathing capacity, and that exchanges oxygen poorly, which results in hypoxaemia. If the disease progresses, it becomes respiratory failure [47]. Pneumocystis pneumonia is often infected in immunocompromised hosts. The dominant symptoms of pneumocystis pneumonia are fever, progressive dyspnea and nonproductive cough [48]. The patient initially has dypsnea on exertion, then later occurs at rest, although orthopnea and nocturnal dypsnea are not features of this disease. The nonproductive cough is irritating. On physical examination, tachypnea is characteristic (respiratory rate over 20 times per minute) and fever over 38 c occurs. The chest is generally clear on auscultation, occasional crepitation being audible. Pneumocystis pneumonia does not involve the pleura or bronchi. Therefore, manifestation of pneumocystis pneumonia are not present as pleuritic chest pain, wheeze or productive cough. AIDS patients who have pneumocystis pneumonia typically present with more insidious onset of respiratory insufficiency, having a median duration about 3-4 weeks, than non AIDS patients [49].

Diagnosis of Pneumocystis Pneumonia

Diagnosis of pneumocystis pneumonia was based on history, physical examination and confirmed by investigation. The chest radiograph characteristically demonstrates bilateral perihilar interstitial infiltrates, which progresses to become more homogeneous and diffuse (Fig. 5). Although in 10% or more of pneumocystis pneumonia cases, the chest radiograph may be entirely normal, and computer tomography scan of the chest may demonstrate extensive ground-glass attenuation [50]. AIDS patients, who have pneumocystis pneumonia, usually present with mild symptoms, modest hypoxaemia and normal chest radiograph [51].

In the past, diagnosis was based on the demonstration of the organisms in material from the lungs or in lung tissue, in suspected cases of pneumocystis pneumonia because *Pneumocystis sp.* cannot be cultured. Sputum induction and bronchoscopy are effective techniques for obtaining specimens, which are used for diagnosis of pneumocystis pneumonia. Thus, the initial procedure for diagnosis of pneumocystis pneumonia should be sputum induction [52], and if that smear is negative, to proceed to bronchoalveolar lavage. Although prophylaxis of pneumocystis pneu-

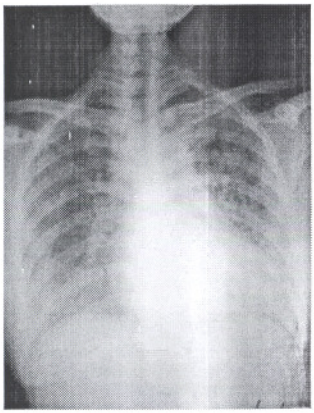

Fig. 5 The posteroanterior chest radiograph of a48-year-old patient with pneumocystis pneumonia demonstrating bilateral perihilar interstitial infiltrates

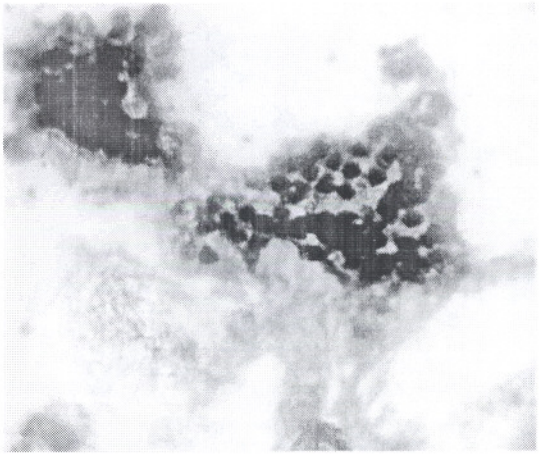

Fig. 6 The Gomori methenamine silver stained-smears, observed at 400 x magnification, shows clusters of *Pneumocystis* cyst are black round to cup-shaped

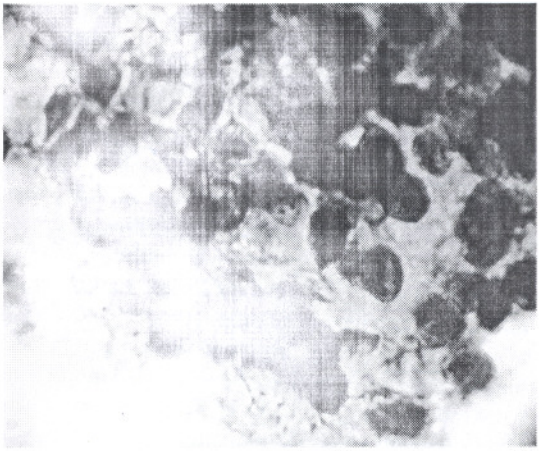

Fig. 7 Cluster of *Pneumocystis* cyst found in Gomori methenamine silver stained-smears, observed at 1,000 x magnification

monia, by aerosolized pentamidine, which reduced organisms in induced sputum specimens, the yield appears to be high enough for the diagnosis of pneumocystis pneumonia in AIDS patients because AIDS patients have more organisms than other immunocompromised patients [53].

Cyst form of *Pneumocystis* organism can be detected by Gomori methenamine silver (GMS) (Fig. 6, 7), Gram-Weigert, or toluidine-blue O stains (Fig. 8, 9). Trophic form can be stained with modified Papanicolaou, Wright-Giemsa, or Gram-Weigert stain. Although *Pneumocystis* organism was seen in cyst form, sporozoite form and trophic form with giemsa stains (Fig. 3, 4), this technique is not sensitive because the contrast of this organism against host cells was not outstanding in these stains [54] (Fig. 6). Calcoflour white chemofluorescent stain is a simple, rapid and inexpensive method for detection of *Pneumocystis* organisms [55]. The application of a mouse monoclonal antibody (2G2), by indirect immunofluorescent assay is sensitive and specific [56]. It can detect both cysts and trophozoite. Physicians who work with AIDS patients need a sensitive, reliable, and noninvasive tool for early detection and diagnosis of pneumonia. Polymerase chain reaction (PCR) that amplify *P. carinii* DNA sequence from bronchoalveolar lavage fluid and induced sputum, are more sensitive and specific than microscopic stain [57]. PCR for detection of *P. carinii* DNA in serologic assays is not of value for diagnosis [58].

Prophylaxis and Treatment

Mild to moderate symptoms of pneumocystis pneumonia can be treated as out-pateints by using oral therapy and close follow up. This decision is based on the clinical status of the patient including the degree of dypsnea, cough, fever, and oxygen saturation, as well as the ability of toleration and compliance with oral therapy [59]. A pateint with significant hypoxaemia should be hospitalized for intravenous therapy. Respiratory failure patient should be admitted to the ICU [60]. Cotrimaxazole, the combination of sulphamethoxazole (SMX) with trimethoprim (TMP), is the first-line drug for the treatment of pneumocystis pneumonia (Table 2). A significant number of patients cannot tolerate the adverse effects of Cotrimaxazole. Alternative therapeutic agents such as atovaquone, trime-thoprim plus dapsone, pentamidine, and clindamycin plus prima-

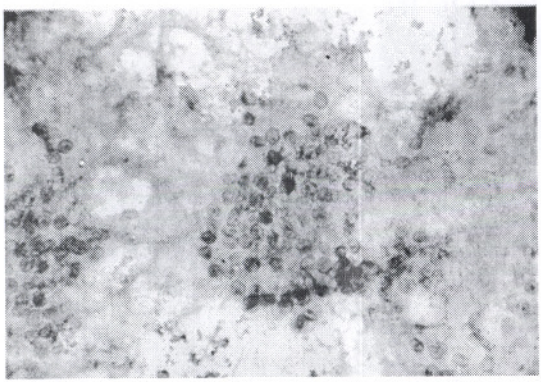

Fig. 8 The toluidine-blue O stained smears, observed at 400 x magnification, show purplish round cyst against the pale green background

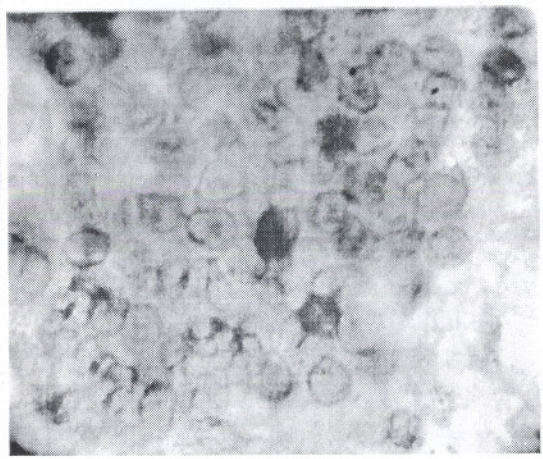

Fig. 9 Cluster of Pneumocystis cysts appear as characteristic disc-like structure. in toluidine-blue O stained smears, observed at 1,000 x magnification

quine are also used (Table 2). Corticosteroids should be given to AIDS patients with pneumocystis pneumonia who have hypoxia (air PaO_2 <70 mmHg within 72 hours of initial therapy while breathing room air) [61].

Cotrimoxazole (SMX-TMP) is also a first-line drug on the prophylaxis of pneumocystis pneumonia, for patients with no history of sulfa allergy (Table 3). Alternative therapeutic agents are Atovaquone, Dapsone, Dapsone plus pyrimethamine (Table 3). Primary prophylaxis should start when CD4+ count is less than 200 cells/millimeter, and when AIDS patients increase CD4+ could be discontinued for 3 months and reintroduced again when CD4+ count falls less than 200 cells/millimeter, or a history of orophyryngeal candidiasis in AIDS patients (including pregnant women) [62]. Lifelong second prophylaxis is recommended for patients who recovered from an episode of pneumocystis pneumonia. Patients receiving immunosuppressive mediations or having an underlying acquired or inherited immunodeficiency without AIDS should receive prophylaxis against pneumocystis pneumonia [63]. Patients who were treated with up to 25 mg. of MTX per week should receive prophylaxis and it if they did not develop severe myelosuppression but they should have close monitoring with CBC and liver function test [64] (Table 3).

Emerging of Drug Resistant Strains of Pneumocystis jirovecii

The enzyme dihydropteroate synthase (DPHS) is the target of sulpha and sulphone drugs in the folic acid pathway. Dihydropteroate synthase (DHPS) catalyses the condensation of *p*-aminobenzoic acid and 6-hydroxymethyl-7, 8-dihydropterin pyrophosphate to form dihydropteroate. DHPS in *P. carinii* is part of a trifunctional protein with two other enzymes

Table 2. Drugs for treatment of pneumocystis pneumonia

Drug	Route	Dose
Drug of choice		
-Trimethoprim-sulfamethoxazole	oral	TMP 15-20 mg/kg/day plus SMX 75-100 mg/kg/day, twice a day
	intravenous	TMP 5 mg/kg plus SMX 25 mg/kg every 8 hours
Alternatives		
-Atovaquone	oral	750 mg twice a day
-Trimethoprim plus dapsone	oral	TMP 5 mg/kg every 8 hours plus dapsone 100 mg daily 4 mg/kg daily
-Pentamidine	intravenous	300-450 mg every 6 hours
-Clindamycin plus primaquine	oral, intravenous	15-30 mg daily
Adjunctive therapy		
-Prednisolone (if room air PaO_2< 70 mmHg within 72 hours of initialing therapy)	oral oral, intravenous	40 mg every 12 hours for 5 days, then 40 mg daily for 5 days, then 20 mg daily for 11 days

Table 3. Drugs for prophylaxis against pneumocystis pneumonia

Drug	Route	Dose
Drug of choice		
-Trimethoprim-sulfamethoxazole	oral	1 double-strength tablet daily or 1 single-strength tablet daily
Alternatives		
-Dapsone	oral	50 mg twice a day or 100 mg daily
-Dapsone plus pyrimethamine plus leucovorin	oral	50 mg daily 50 mg weekly 25 mg weekly
-Dapsone plus pyrimethamine plus leucovorin	oral	200 mg weekly 75 mg weekly 25 mg weekly
-Pentamidine	aerosal	300 mg/kg monthly
-Atovaquone	oral	1,500 mg daily

in folic-acid biosynthesis pathway [65]. Mutation of the gene that encodes dihydropteroate synthase may cause resistance to sulpha agents by decreasing the affinity for sulpha and sulphone drugs. In addition, the mutation of DHPS has been observed in association with the failure of sulpha treatment [66] and prophylaxis and prognosis [65], suggesting that emergence of sulpha drug resistance. Recently, many studies have shown that point mutations in the dihydropteroate synthase (DHPS) gene of human-derived *P. carinii* are related to exposure to sulpha drugs. Mutation of the gene that encodes dihydropteroate synthase may cause resistance to sulpha agents by decreasing the affinity for sulpha and sulphone drugs and possibly represent emergence of sulfa resistance.

Pneumocystis genome projects

International *Pneumocystis* genome project purpose for determining the complete genome sequence of the *P.carinii* and *P.jirovicii*. The genome project will help us to understand more about these organisms. Researchers will discover new knowledge new therapeutic target, evolution differences among the species, which may contribute to the identification of new drug target for prevention and treatment. The *Pneumocystis* genome project plans to finish by early 2005 [67].

Conclusion

Pneumocystis pneumionia is one of the most common opportunistic infections especially in patients with AIDS. Although *Pneumocystis sp.* cannot be cultured, molecular and immunologic approaches help to discover facts of these organisms. *Pneumocystis sp.* is a host-specific organism. The organism that causes human PCP is now named *Pneumocystis jirovecii*, which is now classified as an Archiascomycetous fungus. Diagnosis has been improved by the development of organism-specific monoclonal antibodies and more by polymerase chain reaction. Prophylaxis and treatment failure have been reported for trimethoprim-sulfamathoxazole, considered due to point mutations in dihydropteroate synthase (DHPS). Sequencing of the genome of *Pneumocystis sp.* is going to help us to understand more and more about these organisms.

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