Special Article

Genetic Diversity of *Pneumocystis jirovecii* in Thailand

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A fungus Pneumocystis jirovecii, which causes a diffuse bilateral pneumonia called Pneumocystis pneumonia (PcP) is one of the most common opportunistic infections in HIV-infected patients in Thailand. Molecular techniques have demonstrated diversity among isolates of P. jirovecii by comparison of DNA-sequence variation at the internal transcribed spacer region 1 (ITS1) and region 2 (ITS2) of the nuclear ribosomal RNA genes. The studies confirm that a high diversity of P. jirovecii ITS types exists in different populations from different geographical areas. Type Eg is found globally from represent countries in Europe, North America, South Africa and Asia. Among the 23 types of P. jirovecii observed in Thailand, type Ir is present at the highest frequency (28.6 %), followed by type Eb (21.4%) and types Eg and Rp (14.3 %), respectively. Ir and Rp are unique types observed in Thailand. Mixed infections of more than one types of P. jirovecii are commonly observed in all studies with prevalence of 25-82 %. Moreover, unique types of P. jirovecii can be found in a specific group of populations. These types may be used as genetic markers to study the evolution of the organism in each geographical area.

Keywords: *Pneumocystis jirovecii, Genotypes, Internal transcribed spacer regions*

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Pneumocystis jirovecii (formerly known as *Pneumocystis carinii* special form *hominis* (*P. carinii* f. sp. *hominis*) which originally classified as a protozoan is now reclassified as a fungus by evidences of genetic analysis[1]. *P. jirovecii* causes a diffuse bilateral pneumonia called Pneumocystis pneumonia (PcP), which is pathologically characterized by interstitial plasma cell infiltration with eosinophilic intra-alveolar exudates. PcP was firstly recognized in premature infants and malnourished children in orphanages in Europe after World War II[2]. Ever since, sporadic cases have been reported in patients having organ transplantation, or undergoing chemotherapy for malignant diseases, or congenital immunodeficiencies. Beginning of the epidemic of human immunodeficiency virus (HIV) infection in 1981, the incidence of PcP has increased dramatically and remains the most common opportunistic infection which causes morbidity and mortality in

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HIV-positive patients. In Thailand, PcP which is ranked after tuberculosis, is the second most common opportunistic infection in HIV-infected patients[3]. In North America and Europe, the incidence of PcP has significantly decreased since both highly active antiretroviral therapy (HAART) and the effective anti-*Pneumocystis* chemoprophylaxis have been widely used[4,5]. However, PcP is still the most common life-threatening infection in those who have not yet been diagnosed with HIV or HIV-positive patients not using or not responding to HAART, whose CD4+ T lymphocyte cell count is less than 200 cells/μl. Thus, in countries where HAART and PcP prophylaxis are not widely available to all HIV-positive patients, understanding patterns of transmission are very important for developing methods of intervention.

Recently, a number of genes have been identified for analysis and characterization of P. jirovecii strains. Molecular techniques have demonstrated diversity among isolates of *P. jirovecii* by comparison of DNA-sequence variation at a number of different genetic loci. Molecular typing based on nucleotide sequence variation of *P. jirovecii* genome including the internal transcribed spacer regions 1 (ITS1) and regions 2 (ITS2) of the nuclear ribosomal RNA genes[6-13], the intron of the nuclear 26S ribosomal RNA gene (26S rRNA)[6], the mitochondrial large subunit ribosomal RNA (mtLSU rRNA) gene[14,15], the mitochondrial small subunit ribosomal RNA (mtSSU rRNA) gene[16,17], the dihydropteroate synthase (DHPS) gene and the dihydrofolate reductase (DHFR) gene which encodes a target for the anti-pneumocystis drugs, a combination of trimethroprim and sulfamethoxazole (TMP-SMX) and dapsone[18], the thymidylate synthase (TS) gene[19], the 5S ribosomal RNA gene (5S rRNA)[20], *arom* locus [21] and the β-tubulin (β-tub) gene[22]. Among these genes, mtLSU rRNA, mtSSU rRNA, arom locus, and ITS are DNA targets which have been widely used for sequence analysis. However, the ITS regions of the nuclear ribosomal RNA gene give the most informative typing characteristics since their nucleotide sequences are highly divergent[8,9]. This present review will address the cumulative molecular genetic data of *P. jirovecii* using ITS sequences in different geographical areas including *P. jirovecii* infection in Thai HIV patients attending Phramongkutklao Hospital from 1997 to 2003.

Genetic Differences of Pneumocystis Organisms

Using molecular techniques, heterogeneous group of *Pneumocystis* organisms have been demonstrated. *Pneumocystis* isolated from one species cannot productively infect another species, which means the organism is apparently strict host specificity.

P. jirovecii which infects only human hosts has been shown to be genetically different from other *Pneumocystis* that infect other mammals (rats, mice, shrews, rabbits, ferrets, pigs, horses, monkeys)[23]. The genetic differences of *Pneumocystis* derived from different host species were observed by analysis of chromosomes using gel electrophoresis, which showed that the electrophoretic karyotypes of *Pneumocystis* from rats and humans were similar but distinct[24,25]. Karyotypes from both human-derived and rat-derived *Pneumocystis* contained about 15 bands, ranging in size from 200 to 1,000 kb, but the band patterns of the two kinds of *Pneumocystis* were different. Studies of *Pneumocystis* organisms from ferrets and mice also demonstrated their own electrophoretic karyotypes and the chromosomes from ferret and mouse *Pneumocystis* did not hybridize to any of three genes (DHFR, TS, and β-tubulin genes) from rat Pneumocystis[26]. A similar result was obtained with a gene encoding the major surface glycoprotein (MSG). An MSG gene of *Pneumocystis* from rats hybridized to all bands in an electrophoretic karyotype from rat *Pneumocystis* but did

not hybridize to those from human *Pneumocystis*[27,28]. A 300-bp segment of the mtrRNA sequence has been studied in *Pneumocystis* isolates from nine host species (rats, mice, shrews, rabbits, ferrets, pigs, horses, monkeys, and humans). The sequence variation at this locus ranges between 4 and 27%. The most extensively comparative sequence studies of eight different loci of *Pneumocystis* organisms from humans and rats were performed. The multiloci are different between the two organisms, with variation reaching to 50% at the ITSs region of the nuclear rRNAs gene. These studies confirm that the level of genetic difference between rat-derived *Pneumocystis* and human-derived *Pneumocystis* is greater than those observed between different fungus species of one genus.

Genetic Heterogeneity of *P. jirovecii* in Human Infection

In vitro cultivation of rat-derived *P. carinii* has been demonstrated[29] but the technique has not been successfully applied to *P. jirovecii.* Moreover, small amounts of *P. jirovecii* DNA obtained from clinical samples limit genotyping methods to be done. Molecular tools i.e., DNA amplification using the polymerase chain reaction (PCR) has been useful to study genetic heterogeneity of *P. jirovecii* in clinical samples. Tools for molecular epidemiological studies have been successfully developed to detect levels of genetic heterogeneity within *P. jirovecii.* Studying multiloci genes, sequence polymorphisms have been observed i.e. three nucleotide positions of polymorphism have been reported in a 346-bp portion of the mtLSU rRNA gene[30]. A single base polymorphism has been described at position 81, 85, 248[30]. A low frequency of variation has been observed at position 81 and 248[16] but position 85 is variable at a relatively high frequency. Hence, typing of *P. jirovecii* isolates using the variation at this position has been used in a number of epidemiological studies. The variation has been observed in a 300-bp portion of the gene encoding the mitochondrial small subunit rRNA gene (mtSSU rRNA). A single base polymorphism has been observed at position 160 and position 196[17]. The base A or C has been found at position 160, and T or G at position 196. At present, of the four possible sequence types, only two which are C160/T196 and A160/G196 have been recorded[16]. As a result, a few variations of nucleotides on conserved mtSSU rRNA gene limit molecular typing of *P. jirovecii.*

The enzymes involved in aromatic amino acid biosynthesis in *P. jirovecii* are encoded by the arom gene, which codes for the pentafunctional AROM protein[31]. A 237-bp portion of this gene has been examined for variation among isolates of *P. jirovecii.* Variations at nucleotide position 121 and 208 have been observed. Both these polymorphisms are in the third base position of the codon, encoding valine and serine, respectively, and variation at this position does not change the amino acid sequence. The base C or T has been reported at position 121 and A, G or C at position 208[21]. Of the six possible sequence types, four have been reported which are T121/A208, C121/A208, C121/G208, and C121/C208[16]. The study of the arom gene shows that it is a single copy gene[31]. Thus, the variation at this locus has been used to demonstrate mixed infections with different strains of *P. jirovecii*[21]. Compared with other genetic loci, a high level of sequence divergence has been observed at the internal transcribed spacer regions (ITS) of the nuclear rRNA operon. The ITS1 region is located between the genes encoding the 18S rRNA and the 5.8S rRNA and the ITS2 between the 5.8S rRNA and the 26S rRNA (Fig. 1). The ITS regions are processed by splicing events during the maturation of the rRNA molecules. Differential PCR demonstrated that *P. jirovecii* has only one copy of rRNA gene[32]. A number

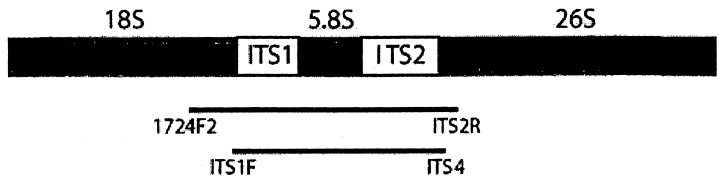

Fig. 1 Locations of ITS regions on nuclear rRNA. The 18S, 5.8S and 26S rRNA genes are represented by black boxes. The ITS regions are illustrated as open boxes. The areas that were amplified from *P. jirovecii* present in human specimens and the primers used for PCR are indicated

of studies have examined diversity in these regions[7,33]. Variations at seventeen positions in the ITS1, together with twenty eight positions of variation at the ITS2 region give a discriminatory power and sensitivity for typing analysis.

Typing of *P. jirovecii using* ITS1 and ITS2 sequences

Scoring methods are available for typing *P. jirovecii* at the ITS regions, i.e., the methods described by Lee et al[8], by Tsolaki et al[16], and by Miller et al[34]. The scoring nucleotide positions reported by Tsolaki et al and by Miller et al provide less discriminatory power than that of Lee et al. Using the ITS region, *P. jirovecii* sequence types are designated with a two-letter code, in which the uppercase letters represent the ITS1 type and the lowercase letters represent the ITS2 type[12]. The ITS1 sequence type are designated types A through R, and the ITS2 are designated types a through r[8,35]. Based on the variations of both ITS regions, Lee et al (1998) reported 15 ITS1 and 14 ITS2 sequence types which can identify 59 types of *P. jirovecii* in 207 clinical specimens collected from 9 countries. Numbers of clinical specimens were approximately 59 % (123 of 207) of PcP patients from Denmark, 21 % (44 of 207) from the United States, and only 14 % (30 of 207) from 7 countries (Ivory Coast, Italy, France, the Netherlands, Portugal, Sweden and Thailand). Due to a relatively small number of samples obtained from most countries, the study focused on identifying types of *P. jirovecii* rather than studying the distribution of types in different geographical areas. Using combinations of ITS1 and ITS2 sequences, types Eg (20.3 %), Ne (14.8 %), and Eb (8.6 %) were present in most countries. Type Eb was described in specimens from Denmark, United States, France, and Portugal. Types Be, Hn, Nb, and Ne were found only in the United States. Lee et al identified types Di, Eg, and Gh in three specimens from Thailand. Type Eg was also the most frequent type described in infants from France[36]. More information of the *P. jirovecii* types have been reported from 19 patients with PcP from Cape Town, South Africa[37]. The most prevalent type was Eg (14 of 19), followed by Gg (4 of 19), Eu (3 of 19) and Gh (2 of 19), respectively. Specific types of Eo, Je, Ge, and No were unique to the South African samples. In Thailand, typing of *P. jirovecii* was conducted in 28 HIV-positive Thai patients attending Phramongkutklao Hospital, Bangkok, Thailand, during 1997-2003[35]. A total of 23 types were identified which were types Ai, Bb, Bi, Bp, Ea, Eb, Ec, Ef, Eg, Eq, Er, Gb, Gg, Ic, Ip, Ir, Jf, Nb, Ne, Rc, Rp, Qb, and Qq, respectively. Among these, thirteen types (Ai, Bb, Bi, Ea, Eb, Ec, Ef, Gb, Jf, Nb, and Ne) were previously reported by Lee et al[8]. The ten new types found in Thai HIV-positive patients were types Bp, Eq, Er, Ic, Ip, Ir, Rc, Rp, Qb, and Qq, respectively. The combination of both ITS1 allele I and ITS2 allele c designated type Ic, was first reported from a PcP patient in Thailand. Among the 23 types of *P. jirovecii*, type Ir is present at the highest

Table 1. *Pneumocystis jirovecii* ITS types found in different countries

Country	ITS types[a]	References
Denmark	Ab, Ac, Ad, Ai, Ba, Bb, Bg, Gh, Bi, Bk, Bm, Cg, De, Dg, Di, Ea, Eb, Ec, Ed, Ee, Ef, Eg*, Eh, Ei, Ej, El, Em, Fg, Fp, Gb, Gg, Gi, He, Ie, Ih, Ii, In, Jf, Kf, Me, Ne, Ng, Ni, Nl, Nn, Oe, Oh, Oh, On	Lee *et al*[8] Helweg-Larsen *et al*[7]
France	Al, Bb, Bi, Bl, Ea, Eb, Ec, Eg*, Ei, El, Em, Fg, Gi, ((H))f, Jf, J((f)), L((f)), Ne, Ng	Lee *et al*[8] Totet *et al*[36]
Italy	Eg, Gf, Hg, Ih, Jf, Jg, Kf*, Of	Lee *et al*[8]
Ivory Coast	Ad, Ai, Bi, Bk, De, Dh, Ec, Ee, Eg, Fg, Gb, Fg, Kf, Li, Me*, Ne, Oe, Og	Lee *et al*[8]
Portugal	Al, Bl*, Eb	Lee *et al*[8]
South Africa	Ea, Eb, Eg*, Eo, Eu, Gb, Ge, Gg, Eh, Ig, Je, Ne, Ng, No	Robberts *et al*[37]
Sweden	Eg*, Na, Ne, Og	Lee *et al*[8]
Thailand	Ai, Bb, Bi, Bp$^+$, Di, Ea, Eb, Ec, Ef, Eg, Eq$^+$, Er$^+$, Gb, Gg, Gh, Ic$^+$, Ip$^+$, Ir*$^+$, Jf, Nb, Ne, Rc$^+$, Rp$^+$, Qb$^+$, Qq$^+$	Lee *et al*[8] Siripattanapipong *et al*[35]
The Netherlands	Ai*, Ne, Oi	Lee *et al*[8]
United States	Ai, Al, Bb, Be, Bi, Eb, Ec, Ee, Eg, Eh, Gg, Hh, Kf, Li, Me, Nb, Nc, Ne*, Ng, Oe, Og	Lee *et al*[8]

[a]*P. jirovecii* ITS type identification by using the score of Lee et al[8]. *, the most common type found in each country. +, type newly described in Thailand

frequency (28.6 %), followed by type Eb (21.4%) and types Eg and Rp (14.3 %), respectively. Ir and Rp are unique types observed in Thailand. The variation of *P. jirovecii* ITS types found in various countries was summarized in Table 1.

All studies confirm that a high diversity of *P. jirovecii* ITS types exists in different populations from different geographical areas. To date, Eg is the most common type found worldwide from countries in Europe, North America, South Africa and Asia. Moreover, unique types of *P. jirovecii* can be found in a specific group of populations. These types may be used as genetic markers to study the evolution of the organism in each area. Additionally, mixed infections of more than one type of *P. jirovecii* are commonly observed in all studies with a prevalence of 25-82 %[8,35-38]. A single copy of ribosomal RNA (rRNA) has been reported in *P. jirovecii*[32], therefore a single transcription occurs. Since multiple types of *P. jirovecii* are commonly described, events of recombination could contribute to the degree of heterogeneity observed in all studies[37].

Usefulness of molecular typing of *P. jirovecii*

Genotyping of the organism permits extensive epidemiological studies. Both the environment and humans have been proposed as the source of *P. jirovecii.* Molecular techniques have been used to determine where DNA of *P. jirovecii* can be found in the environment (i.e. air, soil). Environment samplings gave the evidence that the airborne transmission of *P. jirovecii* is possible. *P. jirovecii* was detected in location where the PcP patients had lived and have been living, such as patient rooms and clinics[34]. Transmission pattern of PcP in persons with AIDS has been studied using the pattern of allelic variation. The evidence of *P. jirovecii* DNA can be detected in the respiratory tract of immunocompetent adults, suggesting humans could be a reservoir and source of the infection[39]. Molecular typing of

P. jirovecii can be used to reveal whether there is any epidemiological link between cases harboring *P. jirovecii* and those from the human reservoir. Transmission of *P. jirovecii* to health care workers in close occupation contact with PcP patients was demonstrated. They became asymptomatic carriage and colonized with the same types of *P. jirovecii* as those found in the patients[34]. However, *P. jirovecii* genotypes isolated from patients with active PcP to susceptible patients caused only a few, indicated that person-to-person transmission may occur but not be the major route of transmission in humans[40].

Typing of *P. jirovecii* is also useful for studying recurrent episodes of PcP. Several studies demonstrated a different *P. jirovecii* type in separate disease episodes[8,12,13,41,42] which prove that the subsequent infections are not necessarily relapses. However, Tsolaki and colleages[13] reported that recurrence caused by the same type of *P. jirovecii* could possibly occur. Other studies showed that genotype frequency distribution patterns varied by the place of residence rather than the place of birth[43]. This likely indicates that the infection is acquired later than the first year of life and that any latency has natural limits[43].

On the basis of ITS sequence information, application of molecular phylogenetic analysis has been used to resolve questions relating to the evolution of the organism. Molecular phylogeny of *P. jirovecii* ITS of RNA gene in Thai HIV-infected patients has been studied to elucidate the evolutionary relationships and molecular epidemiology among genotypes of *P. jirovecii* in Thailand and around the world. Molecular genetic studies showed that the 10 new types found in Thailand is unique information with regard to the molecular epidemiology among HIV-infected patients throughout Europe, America and South Africa[8,37]. Some of the new genotypes such as the type Bp had already coexisted in Thailand. The types Ir, Rp and the new genotype Rp has the potential to reemerge and cause major epidemics of *Pneumocystis* pneumonia in Thai HIV-infected patients. More molecular genetic data will provide not only the patterns of transmission and developing methods of intervention but also the control and prevention strategies of *P. jirovecii.* Moreover, the variable and conserved regions within the ITS regions and 5.8S rRNA gene can be exploited to develop specific probes that can be used as selective amplification primers offering an alternative approach for the rapid identification of a large number of *P. jirovecii* specimens in Thailand in the near future.

References

1. Edman JC, Kovacs JA, Masur H, Santi DV, Elwood HJ, Sogin ML. Ribosomal RNA sequence shows *Pneumocystis carinii* to be a member of the fungi. Nature 1988; 334: 519-22.
2. Cushinon MT. Transmission and epidemiology. In: Walzer PD, editor. *Pneumocystis carinii* pneumonia, 2nd ed. New York: Marcel Dekker; 1994: 123-37.
3. Ruxrungtham K, Phanuphak P. Update on HIV/AIDS in Thailand. J Med Assoc Thai 2001; 84 (Suppl 1): S1-17.
4. Kaplan JE, Hanson D, Dworkin MS, Frederick T, Bertolli J, Lindegren ML, et al. Epidemiology of human immunodeficiency virus-associated opportunistic infections in the United States in the era of highly active antiretroviral therapy. Clin Infect Dis 2000; 30 (Suppl 1): S5-14.
5. Palella FJ Jr, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. N Engl J Med 1998; 338: 853-60.

6. Hauser PM, Francioli P, Bille J, Telenti A, Blanc DS. Typing of *Pneumocystis carinii* f.sp. *hominis* by single-strand conformation polymorphism of four genomic regions. J Clin Microbiol 1997; 35: 3086-91.
7. Helweg-Larsen J, Lee CH, Jin S, Hsueh JY, Benfield TL, Hansen J, et al. Clinical correlation of variations in the internal transcribed spacer regions of rRNA genes in *Pneumocystis carinii* f.sp. *hominis*. AIDS 2001; 15: 451-9.
8. Lee CH, Helweg-Larsen J, Tang X, Jin S, Li B, Bartlett MS, et al. Update on *Pneumocystis carinii* f. sp. *hominis* typing based on nucleotide sequence variations in internal transcribed spacer regions of rRNA genes. J Clin Microbiol 1998; 36: 734-41.
9. Latouche S, Ortona E, Mazars E, Margutti P, Tamburrini E, Siracusano A, et al. Biodiversity of *Pneumocystis carinii hominis*: typing with different DNA regions. J Clin Microbiol 1997; 35: 383-7.
10. Jiang B, Lu JJ, Li B, Tang X, Bartlett MS, Smith JW, et al. Development of type-specific PCR for typing *Pneumocystis carinii* f. sp. *hominis* based on nucleotide sequence variations of internal transcribed spacer region of rRNA genes. J Clin Microbiol 1996; 34: 3245-8.
11. Latouche S, Poirot JL, Bernard C, Roux P. Study of internal transcribed spacer and mitochondrial large-subunit genes of *Pneumocystis carinii hominis* isolated by repeated bronchoalveolar lavage from human immunodeficiency virus-infected patients during one or several episodes of pneumonia. J Clin Microbiol 1997; 35: 1687-90.
12. Lu JJ, Bartlett MS, Shaw MM, Queener SF, Smith JW, Ortiz-Rivera M, et al. Typing of *Pneumocystis carinii* strains that infect humans based on nucleotide sequence variations of internal transcribed spacers of rRNA genes. J Clin Microbiol 1994; 32: 2904-12.
13. Tsolaki AG, Miller RF, Underwood AP, Banerji S, Wakefield AE. Genetic diversity at the internal transcribed spacer regions of the rRNA operon among isolates of *Pneumocystis carinii* from AIDS patients with recurrent pneumonia. J Infect Dis 1996; 174: 141-56.
14. Wakefield AE, Pixley FJ, Banerji S, Sinclair K, Miller RF, Moxon ER, et al. Detection of *Pneumocystis carinii* with DNA amplification. Lancet 1990; 336: 451-3.
15. Wakefield AE, Fritscher CC, Malin AS, Gwanzura L, Hughes WT, Miller RF. Genetic diversity in human-derived *Pneumocystis carinii* isolates from four geographical locations shown by analysis of mitochondrial rRNA gene sequences. J Clin Microbiol 1994; 32: 2959-61.
16. Tsolaki AG, Beckers P, Wakefield AE. Pre-AIDS era isolates of *Pneumocystis carinii* f. sp. hominis: high genotype similarity with contemporary isolates. J Clin Microbiol 1998; 36: 90-3.
17. Hunter JA, Wakefield AE. Genetic divergence at the mitochondrial small subunit ribosomal RNA gene among isolates of *Pneumocystis carinii* from five mammalian host species. J Eukaryot Microbiol 1996; 43: 24S-25S.
18. Ma L, Imamichi H, Sukura A, Kovacs JA. Genetic divergence of the dihydrofolate reductase and dihydropteroate synthase genes in *Pneumocystis carinii* from 7 different host species. J Infect Dis 2001; 184: 1358-62.
19. Edman U, Edman JC, Lundgren B, Santi DV. Isolation and expression of the *Pneumocystis carinii* thymidylate synthase gene. Proc Natl Acad Sci U S A 1989; 86: 6503-7.
20. Kitada K, Oka S, Kimura S, Shimada K, Serikawa T, Yamada J, et al. Detection of

Pneumocystis carinii sequences by polymerase chain reaction: animal models and clinical application to noninvasive specimens. J Clin Microbiol 1991; 29: 1985-90.
21. Banerji S, Lugli EB, Miller RF, Wakefield AE. Analysis of genetic diversity at the arom locus in isolates of *Pneumocystis carinii.* J Eukaryot Microbiol 1995; 42: 675-9.
22. Wakefield AE. Genetic heterogeneity in *Pneumocystis carinii*: an introduction. FEMS Immunol Med Microbiol 1998; 22: 5-13.
23. Revised nomenclature for *Pneumocystis carinii.* The Pneumocystis Workshop. J Eukaryot Microbiol 1994; 41: 121S-122S.
24. Hong ST, Steele PE, Cushion MT, Walzer PD, Stringer SL, Stringer JR. *Pneumocystis carinii* karyotypes. J Clin Microbiol 1990; 28: 1785-95.
25. Zhang J, Cushion MT, Stringer JR. Molecular characterization of a novel repetitive element from *Pneumocystis carinii* from rats. J Clin Microbiol 1993; 31: 244-8.
26. Weinberg GA, Durant PJ. Genetic diversity of *Pneumocystis carinii* derived from infected rats, mice, ferrets, and cell cultures. J Eukaryot Microbiol 1994; 41: 223-8.
27. Stringer JR, Stringer SL, Zhang J, Baughman R, Smulian AG, Cushion MT. Molecular genetic distinction of *Pneumocystis carinii* from rats and humans. J Eukaryot Microbiol 1993; 40: 733-41.
28. Stringer SL, Garbe T, Sunkin SM, Stringer JR. Genes encoding antigenic surface glycoproteins in *Pneumocystis* from humans. J Eukaryot Microbiol 1993; 40: 821-6.
29. Merali S, Frevert U, Williams JH, Chin K, Bryan R, Clarkson AB Jr. Continuous axenic cultivation of *Pneumocystis carinii.* Proc Natl Acad Sci U S A 1999; 96: 2402-7.
30. Sinclair K, Wakefield AE, Banerji S, Hopkin JM. *Pneumocystis carinii* organisms derived from rat and human hosts are genetically distinct. Mol Biochem Parasitol 1991; 45: 183-4.
31. Banerji S, Wakefield AE, Allen AG, Maskell DJ, Peters SE, Hopkin JM. The cloning and characterization of the arom gene of *Pneumocystis carinii.* J Gen Microbiol 1993; 139: 2901-14.
32. Tang X, Bartlett MS, Smith JW, Lu JJ, Lee CH. Determination of copy number of rRNA genes in *Pneumocystis carinii* f. sp. *hominis.* J Clin Microbiol 1998; 36: 2491-4.
33. Liu Y, Rocourt M, Pan S, Liu C, Leibowitz MJ. Sequence and variability of the 5.8S and 26S rRNA genes of *Pneumocystis carinii.* Nucleic Acids Res 1992; 20: 3763-72.
34. Miller RF, Ambrose HE, Wakefield AE. *Pneumocystis carinii* f. sp. hominis DNA in immunocompetent health care workers in contact with patients with P. carinii pneumonia. J Clin Microbiol 2001; 39: 3877-82.
35. Siripattanapipong S, Worapong J, Mungthin M, Leelayoova S, Tan-ariya P. Genotypic study of *Pneumocystis jirovecii* in human immunodeficiency virus-positive patients in Thailand. J Clin Microbiol 2005; 43: 2104-10.
36. Totet A, Pautard JC, Raccurt C, Roux P, Nevez G. Genotypes at the internal transcribed spacers of the nuclear rRNA operon of *Pneumocystis jiroveci* in nonimmunosuppressed infants without severe pneumonia. J Clin Microbiol 2003; 41: 1173-80.
37. Robberts FJ, Liebowitz LD, Chalkley LJ. Genotyping and coalescent phylogenetic analysis of *Pneumocystis jiroveci* from South Africa. J Clin Microbiol 2004; 42: 1505-10.
38. Manoloff ES, Francioli P, Taffe P, Van Melle G, Bille J, Hauser PM. Risk for *Pneumocystis*

carinii transmission among patients with pneumonia: a molecular epidemiology study. Emerg Infect Dis 2003; 9: 132-4.
39. Medrano FJ, Montes-Cano M, Conde M, de la Horra C, Respaldiza N, Gasch A, et al. *Pneumocystis jirovecii* in general population. Emerg Infect Dis 2005; 11: 245-50.
40. Helweg-Larsen J, Tsolaki AG, Miller RF, Lundgren B, Wakefield AE. Clusters of *Pneumocystis carinii* pneumonia: analysis of person-to-person transmission by genotyping. QJM 1998; 91: 813-20.
41. Keely SP, Stringer JR, Baughman RP, Linke MJ, Walzer PD, Smulian AG. Genetic variation among *Pneumocystis carinii* hominis isolates in recurrent pneumocystosis. J Infect Dis 1995; 172: 595-8.
42. Keely SP, Stringer JR. Sequences of *Pneumocystis carinii* f. sp. *hominis* strains associated with recurrent pneumonia vary at multiple loci. J Clin Microbiol 1997; 35: 2745-7.
43. Beard CB, Carter JL, Keely SP, Huang L, Pieniazek NJ, Moura IN, et al. Genetic variation in *Pneumocystis carinii* isolates from different geographic regions: implications for transmission. Emerg Infect Dis 2000; 6: 265-72.

ชนิดสายพันธุ์ของ Pneumocystis jirovecii ในประเทศไทย

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เชื้อรา Pneumocystis jirovecii ทำให้เกิดโรคปอดอักเสบในผู้ป่วยที่มีภาวะภูมิคุ้มกันบกพร่อง โดยในประเทศไทยพบว่าเป็นโรคติดเชื้อฉวยโอกาสที่พบได้บ่อยที่สุดโรคหนึ่งในผู้ป่วยที่ติดเชื้อ HIV จากการศึกษาสายพันธุ์ของเชื้อโดยหารูปแบบความแตกต่างของลำดับเบสของ DNA บริเวณ internal transcribed spacer (ITS1 และ ITS 2) ของจีน rRNA จากจำนวนสายพันธุ์ที่ตรวจพบทั้งหมด 23 สายพันธุ์ พบสายพันธุ์ Ir มากที่สุด (28.6%) รองลงมาคือสายพันธุ์ Eb (21.4%) สายพันธุ์ Eg และสายพันธุ์ Rp (14.3%) ตามลำดับ สายพันธุ์ Ir และสายพันธุ์ Rp เป็นสายพันธุ์ที่พบเฉพาะในประเทศไทย เมื่อเปรียบเทียบกับสายพันธุ์ที่สำรวจในทวีปอื่น ๆ พบว่าสายพันธุ์ Eb สามารถตรวจพบโดยทั่วไปจากทุกประเทศที่มีการสำรวจจากทวีปยุโรป อเมริกาเหนือ อเมริกาใต้ และเอเชีย และการติดเชื้อในผู้ป่วยส่วนใหญ่พบว่าติดเชื้อมากกว่าหนึ่งสายพันธุ์ (25-82%) ซึ่งจากการสำรวจจะพบสายพันธุ์เฉพาะในแต่ละประเทศที่ทำการศึกษา ดังนั้นความแตกต่างที่พบดังกล่าวอาจนำมาใช้เป็น genotypic marker เพื่อใช้ในการศึกษาทางด้านระบาดวิทยาและการติดต่อของเชื้อในแต่ละประเทศได้