Penicilliosis

Kenrad E. Nelson, Khuanchai Supparatpinyo, and Nongnuch Vanittanakom

More than 200 species of the *Penicillium* genus have been described. *Penicillium* organisms are abundant in nature and are common laboratory contaminants. However, *Penicillium marneffei* is the only dimorphic species. The organism is commonly responsible for disseminated invasive infections in humans with HIV infection or AIDS in the endemic areas of Southeast Asia and southern China. *Penicillium marneffei* has also been found to cause natural infections in several species of rodents in the endemic areas, and rodents can be infected experimentally.

History

Penicillium marneffei was originally isolated from the liver of a bamboo rat (*Rhizomys sinensis*) at the Pasteur Institute in Dalat, Viet Nam, in 1956. Capponi and colleagues observed the death of bamboo rats due to disseminated infections with *Penicillium* involving their reticuloendothelial system [1]. These investigators inoculated mice with the newly discovered organism, and it was sent to the Pasteur Institute. The fungus was characterized by Segretain and named *Penicillium marneffei* in honor of Dr. Hubert Marneffe, the Director of the Pasteur Institute of Indochina [2]. Subsequently, Segretain became the first known human to be infected with the organism in 1959 when he accidentally stuck his finger with a needle he was using to inoculate a hamster. The clinical manifestations of his infection were a subcutaneous nodule at the site of the inoculation and lymphadenitis involving the draining axillary lymph nodes. The infection responded to treatment with high doses of oral nystatin.

The first natural human infection with *P. marneffei* was reported in 1973 in a 61-year-old US missionary who was suffering from Hodgkin's disease. His infection was discovered when he underwent a staging splenectomy for Hodgkin's disease [3]. The missionary had visited Southeast Asia after Hodgkin's disease had been diagnosed 1 year prior to the splenectomy. At surgery the excised spleen contained a tan nodular mass, 9 cm in diameter with a necrotic center, which grew *P. marneffei* when cultured on Sabouraud dextrose agar at 25°C. The patient survived after being treated with amphotericin B.

The second case of penicilliosis was reported in 1984 in a 59-year-old man who had traveled in Southeast Asia [4]. He had recurrent episodes of hemoptysis, and *P. marneffei* organisms were isolated from his sputum. Also in 1984, five additional cases involving individuals who had been seen at Ramathibodi Hospital in Bangkok, Thailand, between 1974 and 1982 were reported [5]. Eight cases of *P. marneffei* infection were reported from Guangxi province in southern China that had occurred between 1964 and 1983 [6]. Additional cases were recognized from 1985 to 1991 in southern China [7–9]. These patients were not immunocompromised. All cases had occurred prior to the AIDS epidemic in Southeast Asia.

In the late 1980s and early 1990s several reports of disseminated penicilliosis in HIV-infected patients were published; these included patients who were infected in Southeast Asia but whose infections were diagnosed after they returned to the USA or Europe [10–17]. An HIV-positive Congolese physician developed disseminated penicilliosis while he was working at the Pasteur Institute in Paris [18]. The organism had not been handled directly by the physician, but organisms were being cultured in the building where he was attending a course. This case illustrates the potential hazard of laboratory-acquired infection and suggests an airborne route of infection.

As the HIV/AIDS pandemic has spread in Southeast Asia, *P. marneffei* infection has become a very common opportunistic infection in HIV-infected patients in the region [19–21]. Infection with this organism is now the fourth most common opportunistic infection in AIDS patients in northern Thailand, exceeded only by tuberculosis, *Pneumocystis jiroveci* pneumonia, and cryptococcosis [21]. A total of 550 cases of penicilliosis and 743 cases of cryptococcosis were diagnosed at

K.E. Nelson (✉)
Departments of Epidemiology, Bloomberg School of Public Health, International Health and Medicine, Johns Hopkins University, Baltimore, MD, USA
e-mail: kenelson@jhsph.edu

C.A. Kauffman, et al. (eds.), *Essentials of Clinical Mycology*,
DOI 10.1007/978-1-4419-6640-7_23, © Springer Science+Business Media, LLC 2011

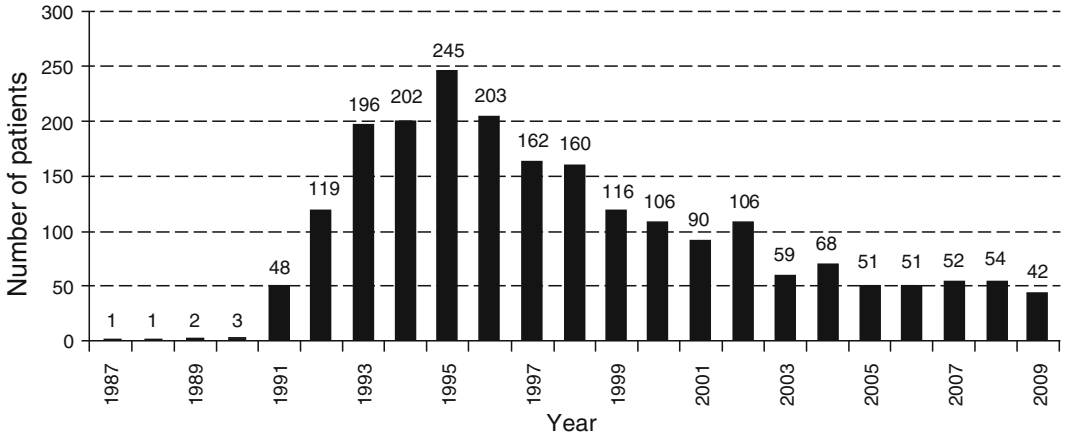

Fig. 1 Number of patients with HIV and *Penicillium marneffei* infection at Chiang Mai Hospital from 1987 to 2009

Chiang Mai University Hospital in northern Thailand between 1991 and 1994. Nearly all of these patients were HIV positive [22]. The number of patients with proven *P. marneffei* infection has declined in the last few years because of a decreased incidence of HIV and widespread availability of antiretroviral treatment (Fig. 1). The endemic area includes Thailand, southern China, Hong Kong, Taiwan, Burma, Laos, Vietnam, Malaysia, and northeast India.

Epidemiology

The natural reservoir of *P. marneffei* is almost certainly the soil. However, the organism was first isolated from Chinese bamboo rats, *Rhizomys sinensis*, in Vietnam in 1956 [1]. Since the original isolation, several investigators in China and Southeast Asia have cultured rodents and environmental samples in order to better understand the reservoir. The organism has been isolated from the internal organs of four species of bamboo rats in Asia (Table 1). Two investigators reported data from bamboo rats collected from Guangxi province in China. Deng and colleagues isolated *P. marneffei* from the internal organs of 18 of 19 *R. pruinosus* rats [24], and Li and colleagues found the organism in 15 of 16 *R. pruinosus* rats [8]. These infected animals showed no signs of illness. However, fatal infections had been observed in bamboo rats that were experimentally infected in Vietnam in 1956 [1, 29]. In another survey in Guangxi province in China, workers isolated *P. marneffei* from 39 of 43 bamboo rats (37 of 41 *R. pruinosus* and 2 of 2 *R. sinensis* [23]. They were also able to isolate *P. marneffei* from soil samples taken from three burrows of *R. pruinosus* rats and from the feces of three animals. Another survey in southern China isolated *P. marneffei* from 114 of 179 (63.7%) *R.* pruinosus rats [25]. A study of the prevalence of *P. marneffei* infections in bamboo rats in central Thailand was done in 1987, and *P. marneffei* was isolated from 6 of 8 (75%) *R. pruinosus* rats and 6 of 31 (19%) *Cannomys badius* rats [26]. Organisms were cultured from the lungs (83%), liver (33%), and pancreas (33%) of these animals.

The prevalence of *P. marneffei* in bamboo rats from northern Thailand was studied in 75 bamboo rats; *P. marneffei* was isolated from the internal organs of 13 of 14 (92.8%) large bamboo rats, *R. sumatrensis*, and 3 of 10 (30%) reddish-brown small bay bamboo rats, *Cannomys badius* [27]. All 51 grayish black *C. badius* rats were negative on culture. Among the *R. sumatrensis* rats, the fungus was most commonly isolated from lungs (86%), spleen (50%), and liver (29%). The investigators also studied 28 soil samples and 67 environmental samples, which had been collected from the residential areas of patients with clinical *P. marneffei* infection. These samples were evaluated using a modified flotation method combined with mouse inoculation to isolate the fungus from the environmental samples [30]. *Penicillium marneffei* was isolated from one soil sample obtained from a burrow of *R. sumatrensis* rats using this method [27]. The other environmental samples were negative.

It is somewhat curious that the prevalence of *P. marneffei* infection among bamboo rats is very high in the numerous surveys that have been reported in the literature, yet the fungus has not been isolated from any other animal in nature. This observation might reflect the animals that are selected for study, since other species have not been extensively studied. However, in part this finding might also reflect the fact that the range of the two genera of bamboo rats, *Rhizomys* and *Cannomys*, coincides with the environmental soil reservoir of *P. marneffei* [31] (Fig. 2). Furthermore, bamboo rats inhabit remote mountainous areas and have extensive soil contact when they burrow. The common isolation of *P. marneffei* from the lungs of infected animals and the rarity of recovery of the organism from the gastrointestinal tract suggests that *P. marneffei* infection is commonly acquired by these animals by inhaling conidia rather than by ingestion.

Table 1 Prevalence of *Penicillium marneffei* infection in trapped bamboo rats in Asia

Species	Positive/tested (%)	Country	References
Rhizomys sinenis	1/1 (100)	Vietnam	Capponi et al. [1]
(Chinese bamboo rat)	2/2(100)	China	Deng et al. [23]
Rhizomys pruinosus	37/41 (90)	China	Deng et al. [24]
(Hoary bamboo rat)	15/16 (94)	China	Li et al. [8]
	114/179 (64)	China	Wei et al. [25]
	6/8 (75)	Thailand	Ajello et al. [26]
Cannomys badius	6/31 (19)	Thailand	Ajello et al. [26]
(Bay bamboo rat or lesser bamboo rat)	3/61 (5)	Thailand	Chariyalertsak et al. [27]
	10/110 (9)	India	Gugnani et al. [28]
Rhizomys sumatrensis	13/14 (93)	Thailand	Chariyalertsak et al. [27]
(Sumatran bamboo rat)			

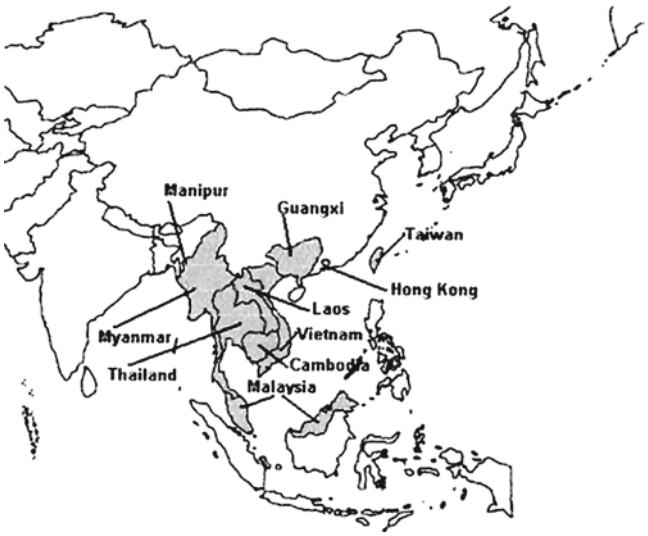

Fig. 2 Endemic areas for *Penicillium marneffei*

In a recent report from India 10 (9.1%) of 110 *C. badius* bamboo rats from Manipur were infected with *P. marneffei*, whereas 72 rodents of other species, including *Bandicota bengalensis*, *Rattus norvegicus*, *Rattus rattus*, *Rattus nitidus*, and *Mus musculus* were all negative. Since these rats were all collected from the same geographic area, and were studied with similar methods, the data suggest that bamboo rats may have increased susceptibility to infection. One bamboo rat isolate had an identical multilocus microsatellite typing pattern to a human isolate from this area [28].

A case-control study compared patients with AIDS who had *P. marneffei* infections to AIDS patients with negative *P. marneffei* cultures in order to help understand the risk factors associated with infection [32]. This study included 80 patients with penicilliosis and 160 control AIDS patients who were admitted to Chiang Mai University Hospital in northern Thailand between December 1993 and October 1995. The main risk factor was occupational soil exposures, especially during the rainy season. Both cases and controls often were familiar with and had seen bamboo rats; 31.3% of cases and 28.1% of controls had eaten bamboo rats but these differences were not significant. The most tenable hypothesis at present is that *P. marneffei* infections, both in humans and bamboo rats, are acquired from a common soil reservoir.

Disseminated *P. marneffei* infections in northern Thailand have been markedly seasonal with a doubling of cases during the rainy season [22]. This seasonality contrasts with *C. neoformans* infection in AIDS patients, which has shown a steady increase during the 1990s as the number of AIDS cases has increased but is not associated with seasonality. This seasonality suggests that many *P. marneffei* infections in AIDS patients may be acquired recently. Also, the environmental reservoir for *P. marneffei* appears to expand during the rainy season. Penicilliosis, while occurring in AIDS patients throughout Thailand, is much more common in the upper northern areas of the country [33]. Whereas penicilliosis accounted for nearly 7.0% of AIDS-defining illnesses in northern Thailand, penicilliosis was seen in only 0.4–1.0% of AIDS patients in other regions of the country. A total of 8,393 patients (2.4%) with disseminated *P. marneffei* infections were reported to the Ministry of Health of Thailand among 358,260 HIV/AIDS cases reported between September 1984 and October 2009 (website, Department of Disease Control, Ministry of Public Health, Thailand).

Organism

Penicillium marneffei grows as a mould on Sabouraud's dextrose agar at 25°C. The mycelial form of the organism is quite variable with green/yellow color with a reddish center. The reverse side of the colony becomes red-brown, and a soluble red pigment diffuses into the agar (Fig. 3). Microscopic examination of the mycelial colony reveals hyaline, septate, branched hyphae with branched conidiophores, or penicilli (Fig. 4). The conidiophores consist of basal stripes with terminal verticils of 3–5 metulae. Each metula has 3–16 phialides. The conidia are oval, smooth-walled, and are 3 μm×2 μm. They are formed basipetally in chains from each phialide. When the organism is transferred to brain–heart infusion agar

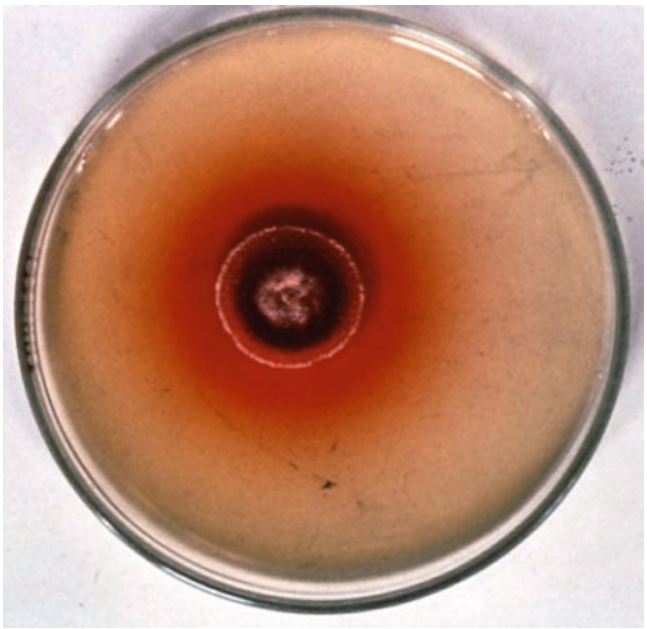

Fig. 3 Mould form of *Penicillium marneffei* plated on Sabouraud's dextrose agar after incubation at 25°C for 5 days, showing the characteristic soluble red pigment that has diffused into the medium

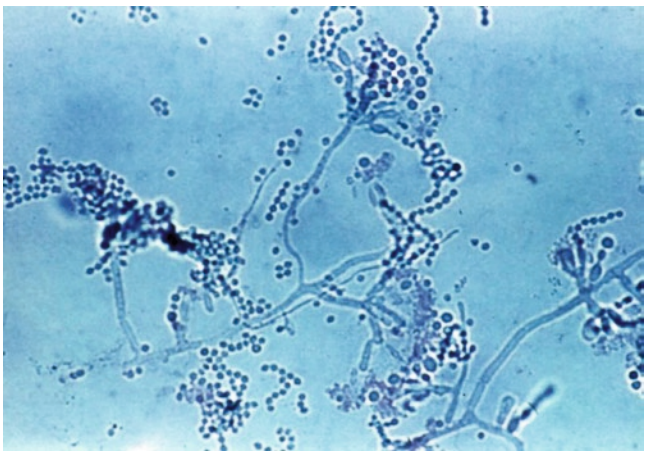

Fig. 4 Mould form of *Penicillium marneffei* grown on Sabouraud's dextrose agar at 25°C. Note the hyaline septate branching hyphae with branched conidiophores, or penicilli, and terminal conidia (600×)

and incubated at 37°C, white to tan-colored colonies of the yeast form develop; no diffusible pigment is produced. Under the microscope the yeasts are unicellular, pleomorphic, elliptical to rectangular cells, which are approximately 2 μm×6 μm in diameter and divide by fission. One or occasionally two septae are seen in the yeast cells.

The organism was first studied in 1959 [2]. *Penicillium marneffei* was originally classified among *Penicillium* species in the section Asymmetrica, subsection of Divaricata in Raper and Thom's taxonomic classification of *Penicillium* species [34]. Pitt later placed *P. marneffei* in the subgenus *Biverticillium* [35]. Recent phylogenetic analysis of nucleotide sequences of nuclear and mitochondrial ribosomal DNA has found that *P. marneffei* is closely related to species of *Penicillium* subgenus *Biverticillium* and sexual *Talaromyces* species with asexual biverticillate states [36]. This genetic analysis allowed the design of unique oligonucleatide primers for the specific amplification of *P. marneffei* DNA.

Penicillium marneffei requires an organic source of nitrogen for mycelial growth. Casein hydrolysate, peptone, and asparagine are utilized, whereas $NaNO_3$ and $(NH_4)_2\ PO_4$ are not. Glucose, lactose, xylose, maltose, laevulose, and mannitol are used as carbon sources. The organism is sensitive to cycloheximide [37]. Investigators have biotyped 32 clinical isolates of *P. marneffei* and found 17 different biotypes [38]. However, none of the biotypes correlated with the clinical characteristics of the infection.

Pathogenesis

Penicillium marneffei infection results from the inhalation of infectious spores or hyphal fragments from the mould form of the organism. At body temperatures (35–37°C), the fungus converts to the yeast form which is disseminated by hematogenous means. The organism primarily infects the reticuloendothelial system, commonly involving liver, spleen, lymph nodes, bone marrow, bone, skin, and lungs. *Penicillium marneffei* conidia bind to the extracellular matrix protein laminin via a sialic acid-dependent process [39]. Also, *P. marneffei* conidia bind to fibronectin, but the binding is less than that to laminin. This binding is also sialic acid-dependent [40]. Similar to other pathogenic dimorphic fungi, the initial host response to *P. marneffei* is histiocytic in nature. The infected histiocytes contain anywhere from a few to many globose to oval yeast cells of *P. marneffei* of fairly uniform size. In the immunocompetent host, the immune response leads to the formation of granulomas that include histiocytes, lymphocytes, plasma cells, and multinucleated giant cells. In patients whose cellular immunity is compromised, tissue necrosis occurs with little or no granuloma formation. Necrotic lesions are surrounded by histiocytes containing yeast cells. Many extracellular yeasts are also present, which are longer and may be irregular in shape compared to intracellular organisms. This histopathologic appearance is common in patients with disseminated penicilliosis. As the infection progresses, the intracellular fungi are released after cellular disruption, and abscess formation and necrosis may occur.

In histologic specimens, neither the cell wall nor the cytoplasm of *P. marneffei* cells takes up hematoxylin eosin stain well. Thus, in routine stained sections, the organisms may appear to be encapsulated. However, the cell walls and septae

are readily stained with Gomori methenamine-silver or periodic acid-Schiff stains. The *P. marneffei* organisms in histiocytes resemble *H. capsulatum* var. *capsulatum*. However, when found extracellularly, *P. marneffei* is usually considerably larger than *H. capsulatum.* The extracellular *P. marneffei* organisms are elongated, sometimes curved, and measure up to 8–13 μm in length. In contrast, yeast cells of *H. capsulatum* var. *capsulatum* are smaller in size, measuring 2–4 μm. By contrast, *H. capsulatum* var. *duboisii* cells are larger, measuring 6–17 μm. *P. marneffei* organisms characteristically contain a single transverse septum and divide by schizogony (fission), whereas *Histoplasma* divide by budding (Table 2).

Chronic latent infections with *P. marneffei* are likely to be common among persons exposed in areas where the organism is endemic. This hypothesis is supported in part by analogy with histoplasmosis pathogenesis and by the long latent periods in some patients between exposure in an endemic area and the onset of clinical infection subsequent to immunosuppression from HIV infection [12, 47]. However, no laboratory methods have been reported to detect latently infected individuals. The development of a skin test or other methods to detect delayed-type hypersensitivity has not been reported for *P. marneffei*. The normal host develops a cell-mediated immune response to *P. marneffei* [23]. The role of T lymphocytes in host defenses against *P. marneffei* has been evaluated in mice experimentally depleted of CD4+ T lymphocytes [48]. These mice developed disseminated infections similar to those seen in AIDS patients. In addition, the in vitro interaction of *P. marneffei* with human leukocytes demonstrated that monocyte-derived macrophages recognize and phagocytose *P. marneffei* even in the absence of opsonization [49]. However, serum factors are required to stimulate TNF-α production. The organisms are able to survive as intracellular pathogens within macrophages. One mechanism of survival is by inhibiting the production of reactive oxygen metabolites or by neutralizing inhibitory host metabolites [50]. The production of acid phosphatase is one of the virulence factors which protects the intracellular *P. marneffei* from the respiratory burst. *Histoplasma capsulatum* has three catalase genes which detoxify hydrogen peroxide [51]. Also, an antigenic catalase-peroxidase protein encoding gene (*cpeA*) in *P. marneffei* was recently isolated by antibody screening of a cDNA yeast-phase library of this organism [52]. The high expression of this *cpeA* gene at 37°C may contribute to the survival of this fungus within host cells. Recently a copper-zinc superoxide dismutase encoding gene has been described and characterized in *P. marneffei* [53]. This polypeptide enzyme has the ability to neutralize toxic levels of reactive oxygen species within the macrophage, thereby allowing the intracellular survival of the organism. Additional research on the sequence of phagocytosis and killing or persistence of *P. marneffei* is needed in order to better understand the natural history and pathogenesis of this infection.

Studies of fungal pathogenesis have included heat shock responses during phase transition as an adaptation response to a higher incubation temperature or to the presence of other noxious stimuli [54, 55]. Recently, *hsp70*, the gene encoding heat shock protein 70 (Hsp70), was cloned and characterized from *P. marneffei* [56]. Expression of *hsp70* is upregulated during temperature-induced and heat shock condition. Moreover, protein profiling of both mould and yeast phases of *P. marneffei* demonstrated the same Hsp70 expression pattern [57, 58]. Expression of a small heat shock protein gene, *P. marneffei* Hsp30, in response to temperature increase was recently reported [59]. A high level of *hsp30* transcript was detected in yeast cells grown at 37°C, whereas a very low or undetectable transcript level was observed in mycelial cells at 25°C. A recombinant Hsp30 protein was produced and tested preliminarily for its immunoreactivity with sera from *P. marneffei*-infected AIDS patients using Western blot analysis. The positive immunoblot result with some serum samples confirmed the antigenic property of the Hsp30. Collectively, the high response of *hsp70* and *hsp30* to temperature increase could indicate that they may play a role in heat stress response and cell adaptation, thereby enabling the parasitic growth of *P. marneffei* in host cells.

Table 2 Microbiologic characteristics of *Penicillium marneffei* and *Histoplasma capsulatum*

Characteristic	*P. marneffei*	*H. capsulatum*
Morphologic features	Biphasic (mould form at 25–30°C, yeast form at 35–37°C)	Biphasic (mould form at 25–30°C, yeast form at 35–37°C)
Distribution	Southern China and Southeast Asia	Worldwide (N. America, S. America, Asia, Africa (*H. duboisii*))
Tissue form	Yeast	Yeast
Size; intracellular (Macrophages)	2–3 × 2–6 μm	2–3 × 2–3 μm
Extracellular	2–3 × 8–13 μm, elongated, curved	Smaller 3–4 μm, diameter
	Septae visible with GMS stain	(*H. duboisii* larger, 6–17 μm diameter)
		No septae
Cell division	Schizogony (fission)	Budding
Specific exoantigen	Positive	Positive

Another possible host-pathogen factor that may play a role in virulence is pigment production. The red pigment of *P. marneffei*, which is synthesized only by the mould phase and is similar to that produced by the nonpathogenic species *Penicillium herquei* [60], is not considered a virulence factor. However, melanins are known virulence factors for many pathogenic fungi [61]. Most fungal melanins are synthesized by either the 3,4-dihydroxy-L-phenylalanine (L-DOPA) or dihydroxynapthalene pathways. Collectively, these dark pigments appear to function in a variety of protective roles, including the inhibition of killing by phagocytes. Like other fungal pathogens, yeast cells of *P. marneffei* have been shown to produce L-DOPA melanin in vivo [62]. Further experimentation will be needed to assess whether melanin may be involved in the virulence of *P. marneffei*.

The collective data described above reveal some insights into the pathogenesis of *P. marneffei* that will need more investigation for the functions of those reported genes or factors involved in phase transition and virulence. Such knowledge may lead to better chemotherapeutic interventions of *P. marneffei* infection.

Clinical Manifestations

Clinically apparent infection with *P. marneffei* occurs most frequently in patients who are severely immunocompromised from an HIV infection. However, infections may also occur in healthy persons or in those immunocompromised for reasons other than HIV/AIDS [63, 64]. Serologic evidence of subclinical infection in a laboratory technician working with the organism has been demonstrated [65]. It is likely that subclinical infections may occur commonly in persons living in endemic areas who are exposed to the organism in nature; however, there is no method to document subclinical infections at present. Disseminated infections have been documented among individuals who have not had contact with areas where the organism is endemic for more than a decade [12].

Typical symptoms and signs of disseminated penicilliosis include fever, malaise, marked weight loss, generalized lymphadenopathy, hepatosplenomegaly, and cough [19, 21]. These nonspecific symptoms are commonly experienced by patients with other chronic infections, such as tuberculosis and other disseminated mycoses. In addition, over 70% of HIV-infected patients with disseminated *P. marneffei* infections present with skin lesions, which are typically symmetrical lesions on the face, chest, and extremities. They appear originally as papules and subsequently become umbilicated, and may become necrotic (Figs. 5–7). Some patients may have smaller, nearly confluent papules, which resemble acne vulgaris or seborrhea. Although skin lesions are more common in patients with *P. marneffei* infection than in those with histoplasmosis or cryptococcosis, the appearance of these lesions is not sufficiently characteristic to be diagnostic. However, a diagnosis can be made by examining a Wright's stain of a skin biopsy or skin smear.

Patients with HIV infection who have disseminated penicilliosis are usually severely immunosuppressed with CD4+ cell counts below 100 cells/μL; the mean CD4+ cell count in one series of cases was 63.8 cells/μL [64]. Disseminated penicilliosis infections have been reported in children with AIDS who lived in an endemic area [66]; however, the incidence appears to be lower in pediatric than in adult AIDS cases, probably because of less frequent exposure to an environmental reservoir among children. One study reported 5 cases of penicilliosis among 157 pediatric AIDS cases diagnosed in northern Thailand [66].

Unusual Clinical Manifestations

As the pandemic of HIV/AIDS spread in Asia and penicilliosis was more widely recognized, an increasing number of patients have been reported with unusual manifestations of *P. marneffei* infections. Patients with chronic lymphadenopathy resembling tuberculous lymphadenopathy have been reported from Hong Kong [67]. Osteomyelitis has been reported in infected adults and may be more common in pediatric patients infected with *P. marneffei* [66, 68]. Some patients have prominent pulmonary symptoms, including localized bronchopulmonary disease, bronchopneumonia,

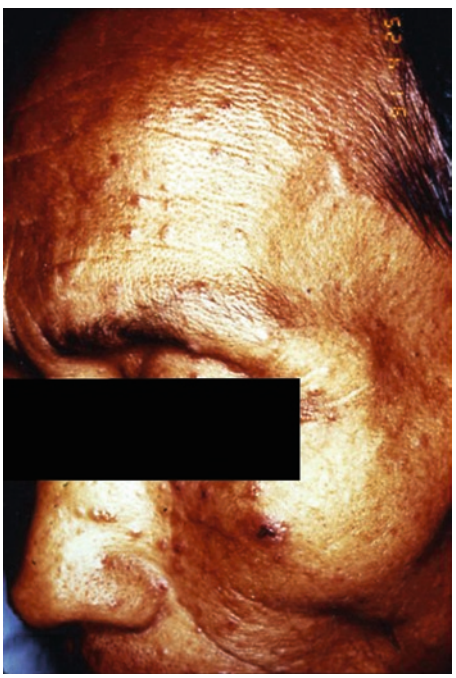

Fig. 5 Patient with disseminated penicilliosis and small papular skin lesions with umbilication and early central necrosis

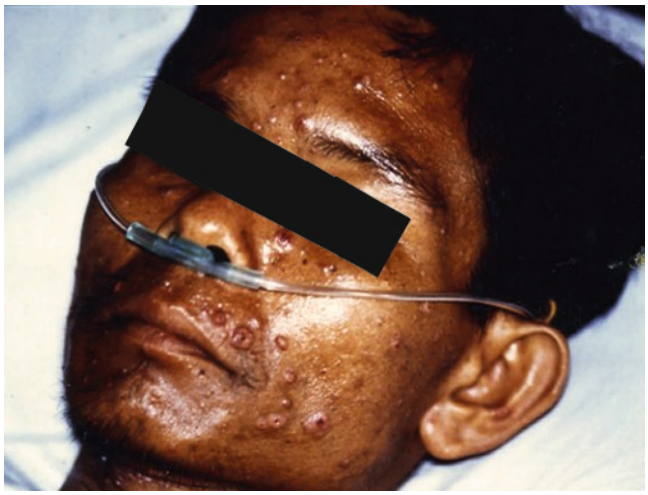

Fig. 6 Young man with disseminated penicilliosis and papulo-umbilicated skin lesions of varying sizes

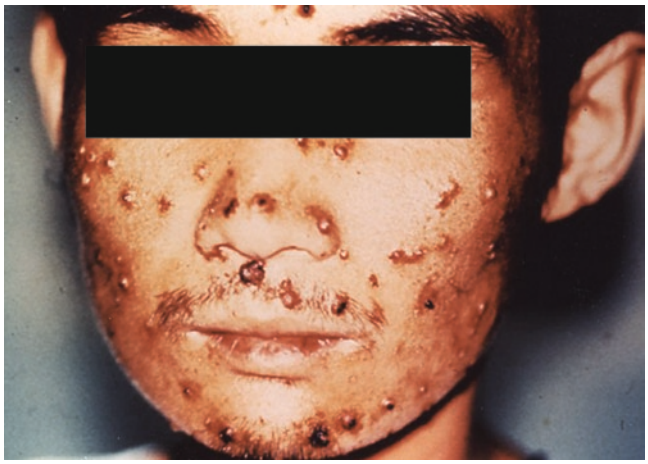

Fig. 7 Papulonecrotic skin lesions seen in a patient with disseminated penicilliosis

cavitary lung disease, and pleural effusions [69, 70]. A retropharyngeal abscess with upper airway obstruction has also been observed [71]. One patient had reactive hemophagocytic syndrome characterized by the proliferation of activated histiocytes throughout the reticuloendothelial system [72]. Rarely, *P. marneffei* has been noted to cause oral [73] and genital ulceration [74].

Penicilliosis in HIV-negative Patients

Although most patients with disseminated penicilliosis infection are severely immunocompromised due to AIDS, some patients are HIV negative. Cooper and Haycocks reviewed 63 penicilliosis cases that had been reported in HIV-negative patients. Twenty-four of the 63 patients (38%) had other conditions predisposing them to a systemic fungal infection. The response to antifungal therapy did not differ substantially whether or not the patients were HIV infected; patients who were untreated had very high mortality rates irrespective of their HIV status [75].

Investigators from Hong Kong compared the clinical and laboratory features of eight HIV-positive and seven HIV-negative patients with penicilliosis [76]. Most of the HIV-negative patients (85.2%) had underlying diseases, including hematologic malignancies, or had received corticosteroids or cytotoxic drugs. The clinical features were not greatly different in the two groups of patients. However, HIV-infected patients had a higher prevalence of fungemia. The investigators, utilizing a *P. marneffei*-specific mannoprotein, Mp1p EIA, found that serum antigen titers were higher in HIV-positive patients, whereas serum antibody levels were higher in HIV-negative patients.

Diagnosis

The diagnosis of penicilliosis rests on the demonstration of the organism in the tissues or the isolation of the organism in cultures from infected patients.

Cultures

The organism grows readily on routine mycologic media, such as Sabouraud dextrose agar or inhibitory mould agar. When cultures are incubated at 25–30°C, *P. marneffei* grows as a mould with typical filamentous reproductive structures of the genus *Penicillium*. The mould form produces a pink or rose-red pigment that diffuses into the medium (Fig. 3). Other *Penicillium* species may also produce a pigment [77]. Therefore, conversion of an organism to the yeast form is required before concluding the isolate is *P. marneffei*. The organism grows as a yeast when incubated at 35–37°C. This form does not produce a red pigment. When incubated at this temperature, the organism undergoes transition into the yeast phase after 12–24 h or so of incubation. The conidia swell and develop into septate hyphae. These hyphae fragment and develop single cells that divide by schizogony (fission). The conversion of the mycelial phase of the organism into the fission yeast phase at higher incubation temperatures is diagnostic of *P. marneffei*. No other *Penicillium* converts to the yeast phase when incubated at 35–37°C. In addition, an exoantigen test for *P. marneffei* has been described, which can also be used to identify cultures of the organism [37].

The organism can be isolated from several sites, including skin, blood, bone marrow, lymph nodes, and sputum. In a population of patients in northern Thailand with disseminated penicilliosis, the organism was isolated from the blood cultures of 76% of 78 patients [20]. However, the blood

cultures were positive for gram-negative bacilli (*Salmonella choleraesuis*, *S. enteritidis*, and *Shigella flexneri*) in 9 of the 19 patients whose cultures did not yield *P. marneffei*. Since these gram-negative organisms grow more rapidly, they could have outgrown the fungus and been responsible for a false-negative culture for *P. marneffei*.

Histopathology

Detection of the organism in biopsies or touch smears of skin lesions or bone marrow aspirates is often possible. A presumptive diagnosis can be made if microscopic examination of a Wright or Giemsa-stained specimen discloses intracellular or extracellular basophilic, spherical, oval, and elliptical yeast-like organisms that are 3–8 μm in diameter, and if the organisms have a clear central septation and are dividing by schizogony (fission) (Fig. 8). *Histoplasma capsulatum* can resemble *P. marneffei*, but *H. capsulatum* divides by budding and is usually smaller. Occasionally *P. marneffei* can be detected in stained smears of peripheral blood [70]. Recently several investigators have reported the identification of *P. marneffei* nucleic acids in clinical specimens as a diagnostic method [78–81].

The use of an exoantigen test has been described for the identification of *P. marneffei* and its differentiation from other species of *Penicillium* [37, 82]; however, the test is not widely used because commercial reagents are not available. Investigators have described the use of a monoclonal antibody in formalin-fixed tissues to detect a specific galactomannan that has an epitope common to *P. marneffei* and *Aspergillus* species [83]. The two invasive fungi must then be differentiated using morphologic criteria. Workers have also reported the use of a specific fluorescent antibody that will differentiate *P. marneffei* from other dimorphic fungi in tissue sections [84].

Serology

Several investigators using different methodologies have reported the detection of antibodies to *P. marneffei* antigens in infected patients. A study in an HIV-infected patient found *P. marneffei* antibodies in serum specimens using immunodiffusion methods with a mycelial phase culture filtrate as antigen [48]. Similar antibodies were found in immunocompetent patients infected with *P. marneffei* [85]. Immunodiffusion has been used to detect antibodies to specific fission arthroconidial filtrate antigens; however, only 2 of 17 *P. marneffei*-infected patients had antibody responses with this assay [86]. An indirect fluorescent antibody test for *P. marneffei* successfully detected antibodies in eight infected patients and was negative in uninfected controls [87]. Serum antibodies were

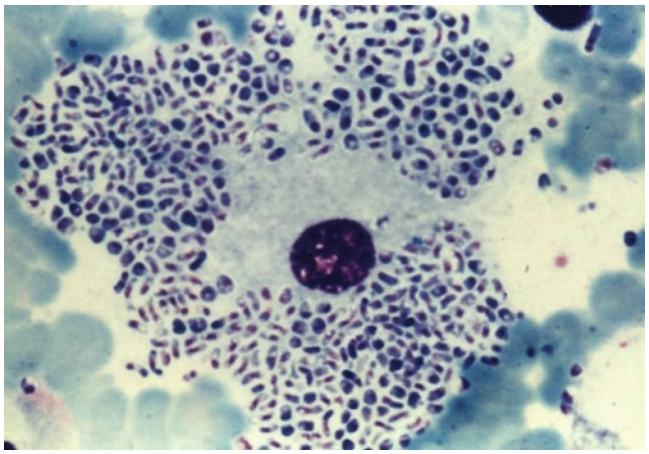

Fig. 8 Bone marrow aspirate showing numerous basophilic bipolar intracellular and extracellular *Penicillium marneffei* organisms in the yeast form. The organisms divide by fission (schizogony) and are 3–8 μm in diameter (Wright's stain, 400×)

detected by ELISA to a purified recombinant mannoprotein of *P. marneffei* in 14 of 17 (82%) HIV-infected patients with documented infection [88]. No false-positive results were found in 90 healthy blood donors, 20 patients with typhoid fever, or 55 patients with tuberculosis.

The protein antigens of yeast and mould phases of *P. marneffei* have been studied by gel electrophoresis and immunoblot assays [89]. More than 20 yeast phase proteins were detected, of which 10 reacted with IgG in the pooled sera of 28 AIDS patients with *P. marneffei* infection. Four immunogenic proteins of 200, 88, 54, and 50 kDa size were produced in large quantity by cultures in the early stationary growth phase. Antibodies to two of these proteins, 54 and 50 kDa, were detected by immunoblot in about 60% of *P. marneffe*-infected AIDS patients but rarely (<5–10%) in AIDS patients without penicilliosis or other controls. One patient's serum was strongly positive 2 months prior to a clinical *P. marneffei* infection, and one asymptomatic laboratory worker working with *P. marneffei* cultures was antibody positive. Further studies of these proteins and a 61-kDa antigen after purification found that 86% of sera from 21 *P. marneffei*-infected patients recognized the 61-kDa, and 71% and 48% recognized the 54-kDa and 50-kDa antigens, respectively [90]. Other investigators have identified a 38-kDa antigen from *P. marneffei* that was recognized by 45% of sera from AIDS patients with penicilliosis [91].

Antigen Detection

Several investigators have described methods to detect *P. marneffei* antigens in serum or urine of infected patients as a method to confirm the diagnosis prior to the isolation of the

organism in culture. Evaluation of immunodiffusion and latex agglutination tests to detect antigenemia in 17 *P. marneffei*-infected patients yielded positive results in 58.8% of infected patients with the immunodiffusion test and 76.5% of patients with the latex agglutination test [86]. Fifteen controls and six patients with cryptococcosis and histoplasmosis were nonreactive. A solid-phase enzyme immunoassay utilizing antibody to *H. capsulatum* var. *capsulatum* to detect *H. capsulatum* antigen in the urine of actively infected patients was cross-reactive with *P. marneffei* in 17 of 18 patients [92]. This assay also was commonly positive in patients with *Blastomyces dermatitidis* and *Paracoccidioides brasiliensis* infections.

Desakorn and colleagues reported the development of a method for quantifying *P. marneffei* antigen in the urine using fluorescein isothiocyanate-labeled purified rabbit hyperimmune immunoglobulin G in an enzyme immunoassay [93]. These investigators studied 33 patients with culture-proven *P. marneffei* infection and 300 controls, including 52 healthy subjects and 248 hospitalized patients in northeast Thailand with a variety of other infections, including melioidosis ($N=168$), other septicemias ($N = 12$), other fungal infections ($N=34$), and miscellaneous conditions ($N = 34$). All of the patients with penicilliosis had measureable antigen in the urine, and in all but two patients, the titers were over 1:40; the median titer was 1:20,480. Whereas 27% of the hospitalized controls and 6% of healthy subjects were positive, the titers were usually below 1:40 in these control groups, leading the investigators to propose a diagnostic cutoff titer of 1:40, which yielded an assay that was 97% sensitive and 98% specific and had a positive predictive value of 84.2% and a negative predictive value of 99.7%.

A follow-up study using this antigen assay in 37 *P. marneffei*-infected patients and 300 controls using ELISA, dot-blot ELISA, or latex agglutination (LA) to detect *P. marneffei* antigen in the urine found sensitivities of 94.6% (dot-ELISA), 97.3% (ELISA), and 100% (LA) and specificities of 97.3–99.3%) [94]. Huang et al. reported that a Platelia *Aspergillus* enzyme immunoassay kit (BioRad) to detect serum galactomannan in patients with *P. marneffei* had a sensitivity of 73.3% [95].

Molecular Diagnosis

Molecular diagnosis in *P. marneffei* is based on specific oligonucleotide primers designed from the internally transcribed spacer and 5.8 S rRNA gene (ITS1-5.8 S-ITS2) of *P. marneffei*. The specificity of these *P. marneffei* primers was tested in a nested PCR [36], and the method was used successfully to identify *P. marneffei* from a skin biopsy [96]. An oligonucleotide probe, based on the 18 S rDNA of *P. marneffei*, has been designed and has proved specific for *P. marneffei* in a PCR-hybridization reaction, regardless of whether the fungus was isolated from humans or natural habitats [97]. This technique could be used to detect *P. marneffei* DNA in EDTA-blood samples collected from AIDS patients with *P. marneffei* infection. Although the method was shown to be highly sensitive and specific, the hybridization technique as described is labor intensive and requires a high level of competence in the laboratory.

To address these concerns, single and nested PCR methods for the rapid identification of *P. marneffei* were then developed using newly designed specific primers, also based on the 18 S rDNA sequence of *P. marneffei* [98]. The sensitivities of single and nested PCR were 1.0 pg/μL and 1.8 fg/μL, respectively, and successful discrimination of a very young culture of *P. marneffei* (2-day-old filamentous colony, 2 mm in diameter) could be performed by the use of this assay. The test has been applied to detect the DNA of *P. marneffei* in patients' serum samples [79, 80] and also in paraffin-embedded tissues [81] from patients and bamboo rats. This PCR method appears to be a valuable, rapid, and complementary technique for the diagnosis of *P. marneffei* infection.

Finally, several investigators have reported methods using restriction enzymes to subtype *P. marneffei* isolates. The use of *Hae*III restriction enzymes to digest *P. marneffei* DNA yielded two DNA profiles (RFLP types I and II) [99]. More recently, the use of *Not*I and pulsed-field gel electrophoresis (PFGE) was used to study the genomic DNA of 64 *P. marneffei* isolates from patients in Thailand [100]. A total of 54 distinct macrorestriction profiles were identified in these patients. Antifungal sensitivity tests, restriction fragment-length polymorphism, and randomly amplified polymorphic DNA patterns in combination have been utilized to subtype 24 strains isolated between 1987 and 1998 from patients in Taiwan [101]. The investigators identified eight highly related patterns and found increased numbers and diversity of strains isolated between 1996 and 1998 compared to those isolated prior to 1996.

Treatment

Disseminated penicilliosis is usually fatal if not treated with appropriate antifungal drugs. However, with early diagnosis and institution of appropriate therapy the mortality rate can be reduced to 10–20% or lower, even among patients with AIDS. Relapse is commonly seen after clinical response among immunocompromised patients unless suppressive doses of antifungal agents are continued.

The in vitro susceptibility of *P. marneffei* to antifungal agents has been evaluated by several investigators (Table 3). A study of 30 clinical isolates from Thailand found that all

Table 3 In vitro drug susceptibility (MIC, range) of *Penicillium marneffei*

References	Number of isolates	Amb	Flu	Itra	Keto	Vor
Jayanetra et al. [5]	3	0.78–3.12	ND	ND	ND	
Sekhon et al. [41]	10	<0.195–1.56	0.195–100	<0.195	0.195–0.39	
Drouhet [42]	10	0.04–1.6	50	≤0.04	≤0.04	
Supparatpinyo et al. [43]	30	0.25–4.0	≤0.313–20	≤0.02–0.078	0.002–0.078	
Imwidthaya et al. [44]	30	0.125–0.5	4.0–8.0	<0.032	<0.125	
Radford et al. [45]	7			≤0.03		≤0.03
Sar et al. [46]	29	0.002–2.0	1.50–256.0	0.002–0.23	0.002–0.19	

Amb amphotericin B, *Flu* fluconazole, *Itra* itraconazole, *Keto* ketoconazole, *Vor* voriconazole

were susceptible to itraconazole, ketoconazole, and miconazole [43]. The organisms were intermediately susceptible to amphotericin B and least susceptible to fluconazole. Some strains were resistant to fluconazole. A study of 29 isolates from AIDS patients in Cambodia and 10 isolates from the lungs of bamboo rats found similar sensitivities to antifungal drugs to the report from Thailand [46]. The in vitro sensitivity of *P. marneffei* to voriconazole is similar to that of itraconazole [45]. Clinical responses to therapy correlate with in vitro susceptibility. Amphotericin B has been shown to be effective in the treatment of disseminated penicilliosis [102]; however, the drug needs to be continued for at least 6–8 weeks. Itraconazole is also effective clinically, but clearance of positive fungal cultures is often delayed for 8 weeks or more [103].

Therapy with voriconazole given intravenously at 6 mg/kg on day 1, 4 mg/kg the next 2 days, and then orally at 200–400 mg twice daily yielded good treatment results in nine of ten evaluable patients [104]. However, the reported experience with voriconazole therapy of *P. marneffei* is limited.

Based upon these clinical results and in vitro data on the antifungal susceptibility of *P. marneffei*, an open-label noncomparative study was done to evaluate the regimen of amphotericin B given intravenously for 2 weeks at 0.6 mg/kg/day followed by itraconazole 400 mg/day taken orally for 10 weeks. This regimen was evaluated in the hope of minimizing the duration and toxicity associated with parenteral amphotericin B while concurrently clearing the fungal cultures more rapidly than with oral itraconazole alone [102]. Of 74 HIV-positive patients with disseminated pencilliosis treated with this regimen, 72 (97.3%) responded. No serious adverse drug effects were observed. After 2 weeks of therapy, 12 patients remained febrile and 11 patients still had skin lesions. By the fourth week of therapy, all patients were afebrile and had resolved their skin lesions. Fungemia was cleared after 2 weeks of treatment in the 65 patients who had a positive blood culture at baseline [102].

Since most patients who present with *P. marneffei* have advanced immunosuppression at the time of diagnosis, initiation of antiretroviral therapy (ART) is recommended in all patients unless there is clear contraindication. The appropriate time for initiation of ART in HIV patients with active opportunistic infection is still controversial. However, ART should be initiated within approximately 2–8 weeks of antifungal therapy in order to match the benefit seen with earlier ART in other opportunistic infections [105].

The immune restoration inflammatory syndrome (IRIS) has been reported uncommonly in HIV patients with *P. marneffei* infection. [106–108] It usually occurs within a few weeks or months after starting ART, suggesting a possibility of immune reconstitution unmasking active disease. Antiretroviral therapy should be continued even if the IRIS occurs. In patients with severe symptomatic IRIS, short-course glucocorticosteroids may be useful [105].

Despite the favorable initial responses to therapy with amphotericin B and itraconazole, relapses are common after antifungal therapy is discontinued in patients with AIDS who have low CD4 counts [43]. Therefore, continued suppressive therapy is required to prevent relapse in patients with disseminated penicilliosis who respond to initial therapy. Suppressive therapy is probably required in AIDS patients for as long as significant immunocompromise persists. In a controlled trial of 71 HIV-infected patients with penicilliosis in Thailand who were not receiving retroviral therapy, 20 (57%) of 35 patients assigned to the placebo group relapsed, whereas none of 36 patients given suppressive itraconazole 200 mg once daily relapsed ($p<0.001$) [109]. The therapy was well tolerated, and the patients in this trial were very compliant with treatment. Suppressive antifungal therapy may be discontinued safely in HIV-infected patients who are treated with ART drugs and respond with clinically significant increases in their CD4 counts [110].

In areas where systemic fungal infections such as *P. marneffei*, *H. capsulatum*, *C. neoformans*, and other fungal infections are common AIDS-associated opportunistic infections, primary prophylaxis against these infections should be considered. In northern Thailand HIV infections are common, involving 2–3% of the general population. Moreover, disseminated fungal infections, especially these due to *P. marneffei*, *C. neoformans*, and *H. capsulatum*, are also common, accounting for over a third of the reported AIDS-defining illnesses in this population [33]. In order to evaluate the efficacy of primary prophylaxis to prevent systemic fungal infection in this population, a clinical trial was done in 129 patients who were HIV positive, had CD4 cell counts <200 cells/μL, and had not experienced a systemic

fungal infection. Patients were randomized to receive oral itraconazole (200 mg/day) or a matched placebo [63]. Systemic fungal infections developed in 1 (1.6%) of 63 patients assigned to itraconazole and 11 (16.7%) of 66 patients assigned to placebo (p = .003). In the placebo group, 7 patients developed cryptococcosis and 4 had penicilliosis. The one patient in the itraconazole group who became infected developed penicilliosis. Clearly, prophylaxis to prevent systemic fungal infections is only necessary in AIDS patients whose HIV infection is not effectively treated with ART. Several clinical trials have clearly shown that patients with systemic pneumocystis [111], cryptococcosis [112], or histoplasmosis [113] are not at risk of relapse of their infection if they have a satisfactory response to HIV therapy.

References

1. Capponi M, Segretain G, Sureau P. Penicillosis from *Rhizomys sinensis*. Bull Soc Pathol Exot Filiales. 1956;49:418–21.
2. Segretain G. Description d'une nouvelle espiece de pencillium: *Penicillium marneffei* n. sp. Bull Soc Mycol France. 1959;75:412–6.
3. DiSalvo AF, Fickling AM, Ajello L. Infection caused by *Penicillium marneffei*: description of first natural infection in man. Am J Clin Pathol. 1973;60:259–63.
4. Pautler KB, Padhye AA, Ajello L. Imported *penicilliosis marneffei* in the United States: report of a second human infection. Sabouraudia. 1984;22:433–8.
5. Jayanetra P, Nitiyanant P, Ajello L, et al. *Penicilliosis marneffei* in Thailand: report of five human cases. Am J Trop Med Hyg. 1984;33:637–44.
6. Deng ZL, Connor DH. Progressive disseminated penicilliosis caused by *Penicillium marneffei*. Report of eight cases and differentiation of the causative organism from *Histoplasma capsulatum*. Am J Clin Pathol. 1985;84:323–7.
7. Li JS, Pan LQ, Deng ZL, Yoo CL. A case report on *Penicillium marneffei*. J Clin Dermatol (China). 1985;14:24–6.
8. Li JS, Pan LQ, Wu SX. Mycologic investigation on *Rhizomys pruinosus* senex in Guangxi as natural carrier with *Penicillium marneffei*. Clin Med J. 1989;102:477–85.
9. Li JS, Pan Q, Wu SX, Su SX, Su B, Shan JH. Diseminated *Penicilliosis marneffei* in China: report of three cases. Clin Med J. 1991;104:247–51.
10. Hilmarsdottir I, Meynard JL, Rogeaux O, Gentilini M. Disseminated *Penicillium marneffei* infection associated with human immunodeficiency virus: a report of two cases and a review of 35 published cases. J Acquir Immune Defic Syndr. 1993;6:466–71.
11. Hulshof CM, van Zanten RA, Sluiters JF, et al. *Penicillium marneffei* infection in an AIDS patient. Eur J Clin Microbiol Infect Dis. 1990;9:370.
12. Jones PD, See J. *Penicillium marneffei* infection in patients infected with human immunodeficiency virus: late presentation in an area of nonendemicity. Clin Infect Dis. 1992;15:744.
13. Kronauer CM, Schar G, Barben M, Buhler H. HIV-associated *Penicillium marneffei* infection. Schweiz Med Wochenschr. 1993; 123:385–90.
14. Peto TE, Bull R, Millard PR, et al. Systemic mycosis due to *Penicillium marneffei* in a patient with antibody to human immunodeficiency virus. J Infect. 1988;16:285–90.
15. Piehl MR, Kaplan RL, Haber MH. Disseminated penicilliosis in a patient with acquired immunodeficiency syndrome. Arch Pathol Lab Med. 1988;112:1262–4.
16. Sobottka I, Albrecht H, Mack D, et al. Systemic *Penicillium marneffei* infection in a German AIDS patient. Eur J Clin Microbiol Infect Dis. 1996;15:256–9.
17. Viviani MA, Tortoranto AM, Rizzardini G, et al. Treatment and serological studies of an Italian case of *Penicilliosis marneffei* contracted in Thailand by a drug addict infected with the human immunodeficiency virus. Eur J Epidemiol. 1993;9:79–85.
18. Hilmarsdottir I, Coutellier A, Elbaz J, et al. A French case of laboratory-acquired disseminated *Penicillium marneffei* infection in a patient with AIDS (letter). Clin Infec Dis. 1994;19:357–8.
19. Nelson KE, Kaufman L, Cooper CR, Merz WG. *Penicillium marneffei:* an AIDS-related illness from Southeast Asia. Infect Med. 1999;16:118–21.
20. Supparatpinyo K, Chiewchanvit S, Hirunsri P, Uthammachai C, Nelson KE, Sirisanthana T. *Penicillium marneffei* infection in patients infected with human immunodeficiency virus. Clin Infect Dis. 1992; 14:871–4.
21. Supparatpinyo K, Khamwan C, Baosoung V, Nelson KE, Sirisanthana T. Disseminated *Penicillium marneffei* infection in southeast Asia. Lancet. 1994;344:110–3.
22. Chariyalertsak S, Sirisanthana T, Supparatpinyo K, Nelson KE. Seasonal variation of disseminated *Penicillium marneffei* infections in northern Thailand: a clue to the reservoir? J Infect Dis. 1996;173:1490–3.
23. Deng Z, Ribas JL, Gibson DW, Connor DH. Infections caused by *Penicillium marneffei* in China and Southeast Asia: review of eighteen published cases and report of four more Chinese cases. Rev Infect Dis. 1988;10:640–52.
24. Deng ZL, Yun M, Ajello L. Human *penicilliosis marneffei* and its relation to the bamboo rat *(Rhizomys pruinosus)*. J Med Vet Mycol. 1986;24:383–9.
25. Wei XG, Ling YM, Li C, Zhang FS. Study of 179 bamboo rats carrying *Penicillium marneffei* (in Chinese). China J Zoonoses. 1987;3:34–5.
26. Ajello L, Padhye AA, Sukroongreung S, Nilakul CH, Tantimavanic S. Occurrence of *Penicillium marneffei* infections among wild bamboo rats in Thailand. Mycopathologia. 1995;131:1–8.
27. Chariyalertsak S, Vanittanakom P, Nelson KE, Sirisanthana T, Vanittanakom N. *Rhizomys sumatrensis* and *Cannomys badius*, new natural animal hosts of *Penicillium marneffei*. J Med Vet Mycol. 1996;34:105–10.
28. Gugnani H, Fisher MC, Paliwal-Johsi A, Vanittanakom N, Singh I, Yadav PS. Role of *Cannomys badius* as a natural animal host of *Penicillium marneffei* in India. J Clin Microbiol. 2004; 42:5070–5.
29. Segretain G. *Penicillium marneffei* n.sp.agent d'une mycose du système reticuloendothelial. Mycopath Mycolgia Appl. 1959; 11:327–53.
30. Vanittanakom N, Mekaprateep M, Sriburee P, Vanittanakom P, Khanjanasthiti P. Efficiency of the flotation method in the isolation of *Penicillium marneffei* from seeded soil. J Med Vet Mycol. 1995; 33:271–3.
31. Corbet GB, Hill JE. Subfamily *Rhizomyinae*: bamboo rats. In: The mammals of the Indo Malaya region: a systemic review. New York: Oxford University Press; 1992. p. 404–7.
32. Chariyalertsak S, Sirisanthana T, Supparatpinyo K, Praparattanapan J, Nelson KE. Case-control study of risk factors for *Penicillium marneffei* infection in human immunodeficiency virus-infected patients in northern Thailand. Clin Infect Dis. 1997;24:1080–6.
33. Chariyalertsak S, Sirisanthana T, Saengwonloey O, Nelson KE. Clinical presentation and risk behaviors of patients with acquired immunodeficiency syndrome in Thailand, 1994–1998: regional variation and temporal trends. Clin Infect Dis. 2001;32:955–62.

34. Raper KB, Thom CA. A manual of the penicillia. Baltimore: The Williams and Wilkins Co; 1949.
35. Pitt JI. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. New York: Academic Press Inc; 1979.
36. LoBuglio KF, Taylor JW. Phylogeny and PCR identification of the human pathogenic fungus *Penicillium marneffei*. J Clin Microbiol. 1995;33:85–9.
37. Sekhon AS, Li JS, Garg AK. *Penicillosis marneffei*: serological and exoantigen studies. Mycopathologia. 1982;77:51–7.
38. Wong SS, Ho TY, Ngan AH, Woo PC, Que TL, Yuen KY. Biotyping of *Penicillium marneffei* reveals concentration-dependent growth inhibition by galactose. J Clin Microbiol. 2001;39:1416–21.
39. Hamilton AJ, Jeavons L, Youngchim S, Vanittanakom N, Hay RJ. Sialic acid-dependent recognition of laminin by *Penicillium marneffei* conidia. Infect Immun. 1998;66:6024–6.
40. Hamilton AJ, Jeavons L, Youngchim S, Vanittanakom N. Recognition of fibronectin by *Penicillium marneffei* conidia via a sialic acid-dependent process and its relationship to the interaction between conidia and laminin. Infect Immun. 1999;67:5200–5.
41. Sekhon AS, Padhye AA, Garg AK. In vitro sensitivity of *Penicillium marneffei* and *Pythium insidiosum* to various antifungal agents. Eur J Epidemiology. 1992;8:427–32.
42. Drouhet E. Penicilliosis due to *Penicillium marneffei*: a new emerging sysemic mycosis in AIDS patients traveling or living in Southeast Asia, Review of 44 cases reported in HIV infected patients during the last 5 years compared to 44 cases of non AIDS patients reported over 20 years. J Mycol Med (Paris). 1993;4:195–224.
43. Supparatpinyo K, Nelson KE, Merz WG, et al. Response to antifungal therapy by human immunodeficiency virus-infected patients with disseminated *Penicillium marneffei* infections and in vitro susceptibilities of isolates from clinical specimens. Antimicrob Agents Chemother. 1993;37:2407–11.
44. Imwidthaya P, Thipsuvan K, Chaiprasert A, Danchaivijitra S, Suthent R, Jearanaisilavong J. *Penicillium marneffei*: types and drug susceptibility. Mycopathologia. 2001;149:109–15.
45. Radford SA, Johnson EM, Warnock DW. In vitro studies of activity of voriconazole (UK-109, 496), a new triazole antifungal agent, against emerging and less-common mold pathogens. Antimicrob Agents Chemother. 1997;41:841–3.
46. Sar B, Boy S, Keo C, et al. In vitro antifungal-drug susceptibilities of mycelial and yeast forms of *Penicillium marneffei* isolates in Cambodia. J Clin Microbiol. 2006;44:4208–10.
47. Hilmarsdottir I, Meynard JL, Rogeaux O, et al. Disseminated *Penicillium marneffei* infection associated with human immunodeficiency virus: a report of two cases and a review of 35 published cases. J Acquir Immune Defic Syndr. 1993;6:466–71.
48. Viviani MA, Tortorano AM, Rizzardini G, et al. Treatment and serological studies of an Italian case of *Penicilliosis marneffei* contracted in Thailand by a drug addict infected with the human immunodeficiency virus. Eur J Epidemiol. 1993;9:79–85.
49. Rongruagruang Y, Levitz SM. Interaction of *Penicillium marneffei* with human leukocytes in vitro. Infect Immun. 1999;67:4732–6.
50. Vanittanakom N, Cooper Jr CR, Fisher MC, Sirisanthana T. *Penicillium marneffei* infection and recent advances in the epidemiology and molecular biology aspects. Clin Microbiol Rev. 2006;19:95–110.
51. Woods JP. *Histoplasma capsulatum* molecular genetics, pathogenesis, and responsiveness to its environment. Fungal Genet Biol. 2002;35:81–97.
52. Pongpom P, Cooper Jr CR, Vanittanakom N. Isolation and characterization of a catalase-peroxidase gene from the pathogenic fungus, *Penicillium marneffei*. Med Mycol. 2005;43:403–11.
53. Thirach S, Cooper Jr CR, Vanittanakom P, Vanittanakom N. The copper, zinc superoxide dismutase gene of *Penicillium marneffei*: cloning, characterization, and differential expression during phase transition and macrophage infection. Med Mycol. 2007;45:409–17.
54. Bastos KP, Bailao AM, Borges CL, et al. The transcriptome analysis of early morphogenesis in *Paracoccidioides brasiliensis* mycelium reveals novel and induced genes potentially associated to the dimorphic process. BMC Microbiol. 2007;7:29.
55. Burnie JP, Carter TL, Hodgetts SJ, Matthews RC. Fungal heat-shock proteins in human disease. FEMS Microbiol Rev. 2006;30:53–88.
56. Kummasook A, Pongpom P, Vanittanakom N. Cloning, characterization and differential expression of an hsp70 gene from the pathogenic dimorphic fungus, *Penicillium marneffei*. DNA Seq. 2007;18:385–94.
57. Chandler JM, Treece ER, Trenary HR, et al. Protein profiling of the dimorphic, pathogenic fungus *Penicillium marneffei*. Proteome Sci. 2008;6:17.
58. Cooper CR, Vanittanakom N. Insights into the pathogenicity of *Penicillium marneffei*. Future Microbiol. 2008;3:43–55.
59. Vanittanakom N, Pongpom M, Praparattanapan J, Cooper Jr CR, Sirisanthana T. Isolation and expression of heat shock protein 30 gene from *Penicillium marneffei*. Med Mycol. 2009;47:521–6.
60. Bhardwaj S, Shukla A, Mukherjee S, et al. Putative structure and characteristics of a red water-soluble pigment secreted by *Penicillium marneffei*. Med Mycol. 2007;45:419–27.
61. Nosanchuk JD, Casadevall A. Impact of melanin on microbial virulence and clinical resistance to antimicrobial compounds. Antimicrob Agents Chemother. 2006;50:3519–28.
62. Youngchim S, Hay RJ, Hamilton AJ. Melanization of *Penicillium marneffei* in vitro and in vivo. Microbiology. 2005;151:291–9.
63. Chariyalertsak S, Supparatpinyo K, Sirisanthana T, Nelson KE. A controlled trial of itraconazole as primary prophylaxis for systemic fungal infections in patients with advanced human immunodeficiency virus infection in Thailand. Clin Infect Dis. 2002;34:277–84.
64. Vanittanakom N, Sirisanthana T. *Penicillium marneffei* infection in patients infected with human immunodeficiency virus. Curr Top Med Mycol. 1997;8:35–42.
65. Vanittanakom N, Merz WG, Nelson KE, Sirisanthana T. Rapid detection of *Penicillium marneffei* by polymerase chain reaction/hybridization technique. 13th Congress of International Society for Human and Animal Mycology, Parma, Italy, 8–13 June 1997.
66. Sirisanthana V, Sirisanthana T. Disseminated *Penicillium marneffei* infection in human immunodeficiency virus-infected children. Pediatr Infect Dis J. 1995;14:935–40.
67. Yuen WC, Chan YF, Loke SL, Seto WH, Poon GP, Wong KK. Chronic lymphadenopathy caused by *Penicillium marneffei*: a condition mimicking tuberculous lympadenopathy. Br J Surg. 1986;73:1007–8.
68. Chan YF, Woo KC. *Penicillium marneffei* osteomyelitis. J Bone Joint Surg Br. 1990;72:500–3.
69. Chan JK, Tsang DN, Wong DK. *Penicillium marneffei* in bronchoalveolar lavage fluid. Acta Cytol. 1989;33:523–6.
70. Supparatpinyo K, Sirisanthana T. Disseminated *Penicillium marneffei* infection diagnosed on examination of a peripheral blood smear of a patient with human immunodeficiency virus infection. Clin Infect Dis. 1994;18:246–7.
71. Ko KF. Retropharyngeal abscess caused by *Penicillium marneffei*: an unusual cause of upper airway obstruction. Otolaryngol Head Neck Surg. 1994;110:445–6.
72. Chim CS, Fong CY, Ma SK, Wong SS, Yuen KY. Reactive hemophagocytic syndrome associated with *Penicillium marneffei* infection. Am J Med. 1998;104:196–7.
73. Tong AC, Wong M, Smith NJ. *Penicillium marneffei* infection presenting as oral ulcerations in a patient infected with human immunodeficiency virus. J Oral Maxillofac Surg. 2001;59:953–6.
74. Annam V, Inamadar AC, Palit A, Koppad M, Peerapur BV, Yelikar BR. Genital ulcer caused by *Penicillium marneffei* in an HIV-infected patient. Sex Transm Infect. 2007;83:249–50.
75. Cooper Jr CR, Haycocks NG. *Penicillium marneffei*: an insurgent species among the penicillia. J Eukaryot Microbiol. 2000;47:24–8.
76. Wong SSY, Wong KH, Hui WT, et al. Differences in clinical and laboratory diagnostics characteristics of *Penicilliosis marneffei* in human immunodeficiency virus (HIV) and non-HIV infected patients. J Clin Microbiol. 2001;39:4534–40.

77. Viviani MA, Tortoranto AM. *Penicillium marneffei*. In: Ajello L, Hay RJ, editors. Topley and Wilson's microbiology and microbial infections. 9th ed. London, UK: Arnold Press; 1998. p. 409–19.
78. Hsiue HC, Huang YT, Kuo YL, Liao CH, Chang TC, Hsueh PR. Rapid identification of fungal pathogens in positive blood cultures using oligonucleotide array hybridization. Clin Microbiol Infect. 2010;16(5):493–500. 15 July 2009 (Epub ahead of print).
79. Pongpom M, Sirisanthana T, Vanittanakom N. Application of nested PCR to detect *Penicillium marneffei* in serum samples. Med Mycol. 2009;47:549–53.
80. Pornprasert S, Praparattanapan J, Khamwan C, et al. Development of TaqMan real-time polymerase chain reaction for the detection and identification of *Penicillium marneffei*. Mycoses. 2009;52:487–92.
81. Zeng H, Li X, Chen X, et al. Identification of *Penicillium marneffei* in paraffin-embedded tissue using nested PCR. Mycopathologia. 2009;168:31–5.
82. Sekhon AS, Garg AK, Padhye AA, Standard PG, Kaufman L, Ajello L. Antigenic relationship of *Penicillium marneffei* to *P. primulinum*. J Med Vet Mycol. 1989;27:105–12.
83. Estrada JA, Stynen D, Cutsem JV, Pierar-Franchimont C, Pierard GE. Immunohistochemical identification of *Penicillium marneffei* by monoclonal antibody. Dermatol. 1992;31:410–2.
84. Kaufman L, Standard PG, Anderson SA, Jalbert M, Swisher BL. Development of specific fluorescent-antibody test for tissue form of *Penicillium marneffei*. J Clin Microbiol. 1995;33:2136–8.
85. Sekhon AS, Stein L, Garg AK, Black WA, Glezos JD, Wong C. Pulmonary *penicillosis marneffei*: report of the first imported case in Canada. Mycopathologia. 1994;128:3–7.
86. Kaufman L, Standard PG, Jalbert M, Kantipong P, Limpakarnjanarat K, Mastro TD. Diagnostic antigenemia tests for *Penicilliosis marneffei*. J Clin Microbiol. 1996;34:2503–5.
87. Yuen K, Wong SS, Tsang DN, Cau PY. Serodiagnosis of *Penicillium marneffei* infection. Lancet. 1994;344:444–5.
88. Cao L, Chen DL, Lee C, et al. Detection of specific antibodies to an antigenic mannoprotein for diagnosis of *Penicillium marneffei* penicilliosis. J Clin Microbiol. 1998;36:3028–31.
89. Vanittanakom N, Mekaprateep M, Sittisombut N, et al. Western immunoblot analysis of protein antigens of *Penicillium marneffei*. J Med Vet Mycol. 1997;35:123–31.
90. Jeavons L, Hamilton AJ, Vanittanakom N, et al. Identification and purification of specific *Penicillium marneffei* antigens and their recognition by human immune sera. J Clin Microbiol. 1998;36:949–54.
91. Chongtrakool P, Chaiyaroj SC, Vithayasai V, et al. Immunoreactivity of a 38-kilodalton *Penicillium marneffei* antigen with human immunodeficiency virus-positive sera. J Clin Microbiol. 1997;35:2220–3.
92. Wheat J, Wheat H, Connolly P, et al. Cross-reactivity in *Histoplasma capsulatum* variety *capsulatum* antigen assays of urine samples from patients with endemic mycoses. Clin Infect Dis. 1997;24:1169–71.
93. Desakorn V, Smith MD, Walsh AL, et al. Diagnosis of *Penicillium marneffei* infection by quantitation of urinary antigen by using an enzyme immunoassay. J Clin Microbiol. 1999;37:117–21.
94. Desakorn V, Simpson AJ, Wuthiekanun V, et al. Development and evaluation of rapid urinary antigen detection tests for diagnosis of *Penicilliosis marneffei*. J Clin Microbiol. 2002;40:3179–83.
95. Huang YT, Hung CC, Liao CH, Sun HY, Chang SC, Chen YC. Detection of circulating galactomannan in serum samples for diagnosis of *Penicillium marneffei* infection and cryptococcosis among patients infected with human immunodeficiency virus. J Clin Microbiol. 2007;45:2858–62.
96. Tsunemi Y, Takahashi T, Tamaki T. *Penicillium marneffei* infection diagnosed by polymerase chain reaction from the skin specimen. J Am Acad Dermatol. 2003;49:344–6.
97. Vanittanakom N, Merz WG, Sittisombut N, Khamwan C, Nelson KE, Sirisanthana T. Specific identification of *Penicillium marneffei* by a polymerase chain reaction/hybridization technique. Med Mycol. 1998;36:169–75.
98. Vanittanakom N, Vanittanakom P, Hay RJ. Rapid identification of *Penicillium marneffei* by PCR-based detection of specific sequences on the rRNA gene. J Clin Microbiol. 2002; 40:1739–42.
99. Vanittanakom N, Cooper Jr CR, Chariyalertsak S, Youngchim S, Nelson KE, Sirisanthana T. Restriction endonuclease analysis of *Penicillium marneffei*. J Clin Microbiol. 1996;34:1834–6.
100. Trewatcharegon S, Sirisinha S, Romsai A, Eampokalap B, Teanpaisan R, Chaiyaroj SC. Molecular typing of *Penicillium marneffei* isolates from Thailand by NotI macrorestriction and pulsed-field gel electrophoresis. J Clin Microbiol. 2001; 39:4544–8.
101. Hsueh PR, Teng LJ, Hung CC, et al. Molecular evidence for strain dissemination of *Penicillium marneffei*: an emerging pathogen in Taiwan. J Infect Dis. 2000;181:1706–12.
102. Sirisanthana T, Supparatpinyo K, Perriens J, Nelson KE. Amphotericin B and itraconazole for treatment of disseminated *Penicillium marneffei* infection in human immunodeficiency virus-infected patients. Clin Infect Dis. 1998;26:1107–10.
103. Supparatpinyo K, Chiewchanvit S, Hirunsri P, et al. An efficacy study of itraconazole in the treatment of *Penicillium marneffei* infection. J Med Assoc Thailand. 1992;75:688–91.
104. Supparatpinyo K, Schlamm HT. Voriconazole as therapy for systemic *Penicillium marneffei* infections in AIDS patients. Am J Trop Med Hyg. 2007;77:350–3.
105. Kaplan JE, Benson C, Holmes KH, Brooks JT, Pau A, Masur H. Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. MMWR Recomm Rep. 2009;58:1–207.
106. Gupta S, Mathur P, Maskey D, Wig N, Singh S. Immune restoration syndrome with disseminated *Penicillium marneffei* and cytomegalovirus co-infections in an AIDS patient. AIDS Res Ther. 2007;4:21.
107. Manosuthi W, Chaovavanich A, Tansuphaswadikul S, et al. Incidence and risk factors of major opportunistic infections after initiation of antiretroviral therapy among advanced HIV-infected patients in a resource-limited setting. J Infect. 2007;55:464–9.
108. Saikia L, Nath R, Biswanath P, Hazarika D, Mahanta J. *Penicillium marneffei* infection in HIV infected patients in Nagaland & immune reconstitution after treatment. Indian J Med Res. 2009;129:333–4.
109. Supparatpinyo K, Perriens J, Nelson KE, Sirisanthana T. A controlled trial of itraconazole to prevent relapse of *Penicillium marneffei* infection in patients infected with the human immunodeficiency virus. N Engl J Med. 1998;339:1739–43.
110. Chaiwarith R, Charoenyos N, Sirisanthana T, Supparatpinyo K. Discontinuation of secondary prophylaxis against *Penicilliosis marneffei* in AIDS patients after HAART. AIDS. 2007;21:365–7.
111. Lopez Bernaldo de Quiros JC, Miro JM, Pena JM, et al. A randomized trial of the discontinuation of primary and secondary prophylaxis against *Pneumocystis carinii* pneumonia after highly active antiretroviral therapy in patients with HIV infection. Grupo de Estudio del SIDA 04/98. N Engl J Med. 2001;344:159–67.
112. Rollot F, Bossi P, Tubiana R, et al. Discontinuation of secondary prophylaxis against cryptococcosis in patients with AIDS receiving highly active antiretroviral therapy. AIDS. 2001; 15:1448–9.
113. Goldman M, Zackin R, Fichtenbaum CJ, et al. Safety of discontinuation of maintenance therapy for disseminated histoplasmosis after immunologic response to antiretroviral therapy. Clin Infect Dis. 2004;38:1485–9.