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Pneumocystis Jirovecii Pneumonia: Current Knowledge and Outstanding Public Health Issues

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Abstract Antiretroviral therapy and cotrimoxazole prophylaxis have caused a decline in *Pneumocystis jirovecii* incidence in developing countries since the 1990s. Nevertheless, *Pneumocystis jirovecii* pneumonia (PCP) remains the primary AIDS-defining disease around the world. Much about the disease remains unknown, including its global burden, best diagnostic practices, the frequency of drug resistance, and the risks associated with *Pneumocystis* colonization. This review describes current knowledge about *Pneumocystis* infection and highlights areas where new research could benefit public health.

Keywords *Pneumocystis* · PCP · *Jirovecii* · *Carinii* · Pneumonia · HIV · Immunosuppressed · Fungal · DHPS · Colonization · Prophylaxis · Oral washes

Introduction

Pneumocystis jirovecii (*P. carinii* until 1999) pneumonia (PCP) is an opportunistic fungal infection affecting immunocompromised persons worldwide. Children are exposed early in life, but immunocompetent hosts usually remain unaffected by disease [1]. During the early to mid 1900s, outbreaks of PCP occurred in Iran and Europe among malnourished children and premature infants [2, 3], but the association between immune dysfunction and PCP remained unrecognized until the 1960s and 1970s, when irradiated cancer patients and immunosuppressed organ transplant recipients began developing the disease [4]. The advent of the AIDS pandemic brought pneumocystosis notoriety in the medical field as a primary AIDS-defining illness. Indeed, the occurrence of PCP among a group of otherwise healthy young men in the early 1980s led astute physicians to seek an underlying etiology consistent with profound immune damage, and was the first description of AIDS [5, 6].

Although the introduction of PCP prophylaxis and antiretroviral therapy (ART) during the 1990s led to substantial reductions in PCP morbidity and mortality among AIDS patients in developed countries [3, 7–9], the disease continues to affect HIV-infected persons not receiving or not responding to ART; HIV-exposed but uninfected children [10]; other persons receiving immunosuppressive therapies, such as transplant patients [3]; persons with connective tissue diseases [11]; and children with chronic lung diseases [12]. It has also been postulated as a cause of mild respiratory disease among normal, healthy infants [13].

Despite its role as a primary AIDS-defining infection, many questions about PCP remain unanswered, including the global burden of disease, the frequency and significance of asymptomatic colonization with the pathogen, disease transmission mechanisms, molecular determinants of virulence and drug resistance, the pathogen's life cycle, and cost-effective diagnostic options. We review the current state of knowledge on PCP and discuss outstanding public health questions that need to be addressed.

Pneumocystis Life Cycle, Reservoir, and Disease Transmission

Three different forms of *Pneumocystis* species are included in the life cycle: trophozoites, sporozoites, and mature cysts [14]. Although details of the life cycle have

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been difficult to elaborate owing to challenges in culturing the organism, ultrastructural studies have informed proposed models of the cycle [14]. According to such models, it is believed that humans become infected through the inhalation of airborne haploid spores, after which sexual conjugation in the lungs produces a diploid cell that undergoes meiosis and subsequent mitosis, resulting in eight nuclei in a single sporocyte (ascospore) [14, 15]. Maturation occurs as the ascospore wall thickens and it becomes a cyst; subsequent cyst rupture liberates the eight spores into the lungs or air, where they can spread infection within the host or infect others [14]. It is known that trophozoite forms represent approximately 90% of the microbe in the lungs of infected hosts [14], but except for rare findings of *Pneumocystis* DNA in airborne fungal spore samples and pond water [16, 17], little is known about the pathogen's existence outside of humans. Other mammals can become infected with organisms related to *Pneumocystis jirovecii*, but infecting *Pneumocystis* species differ widely between mammalian host species [18, 19], and the existence of an environmental or animal reservoir for human infection is unknown [20].

Extensive evidence suggests that *P. jirovecii* is transmissible between humans [21•, 22, 23]. The findings of sulfa-resistant *P. jirovecii* in persons previously unexposed to sulfa drugs, changes in the infecting *P. jirovecii* genotype in patients from one episode of PCP to the next, and findings that infecting strains of *P. jirovecii* are genetically characteristic of the patient's diagnosis location rather than their birth location [24] suggest the occurrence of local person-to-person transmission rather than reactivation of latent childhood infection [25–27]. It has been suggested that, in addition to infected patients, immunocompromised persons carrying *P. jirovecii* asymptomatically might serve as reservoirs for infection of other immunocompromised persons [27].

Pneumocystis Jirovecii Infection

Classic signs and symptoms of *Pneumocystis* infection include dyspnea, a dry cough, rapid breathing, fever, chills, sweats, fatigue, and cyanosis—symptoms that can easily be confused with those of tuberculosis (TB). Disseminated *Pneumocystis* infection is very rare. Immunosuppression, short-term or long-term corticosteroid use, and the presence of chronic lung disease are the primary risk factors for infection with *Pneumocystis* species [3]. In healthy adults, *P. jirovecii* is either absent or present at low levels [28–30]. In immunocompromised adults, the number of *P. jirovecii* in the lungs is directly correlated with the patient's degree of immunosuppression [31].

Clinical algorithms can partially help distinguish PCP among AIDS patients from other common respiratory infections, such as TB. Clinically, differences have been noted between HIV-infected patients with negative acid-fast bacillus (AFB) smears and abnormal chest x-rays who do and do not have PCP [32]: PCP patients have significantly lower oxygen saturation, fewer CD4 cells, greater weight loss, more cyanosis, more severe dyspnea, and higher respiratory rates [32, 33]. These clinical parameters can prove useful in guiding diagnosis, particularly in distinguishing PCP from smear-negative TB.

In addition, PCP presents differently in HIV-infected and HIV-uninfected patients. HIV-infected patients with PCP have subacute onset of progressive dyspnea, a nonproductive cough, and low-grade fever [34]. More acute disease usually suggests an alternative diagnosis. Mortality from PCP among HIV-infected patients has been shown to be about 12%, although the prognosis is worse for patients requiring intensive care [34]. In contrast, onset of PCP among HIV-uninfected persons is more acute and is frequently associated with higher fever, dry cough, and a requirement for mechanical ventilation. Interestingly, although HIV-uninfected patients have many fewer *Pneumocystis* organisms detectable in their lungs than HIV-infected patients, their overall mortality from PCP appears to be higher, approaching 40% [34].

Colonization

Colonization can be defined as the presence of *Pneumocystis* DNA or organisms in the respiratory tract in the absence of clinically apparent infection. Although it is clear that humans can become colonized with *Pneumocystis*, the significance of colonization is unclear. Understanding the relationships between colonization and disease transmission and between colonization and the risk of developing future disease are important because these relationships can guide recommendations, both for treatment of colonized patients and for the protection of immunosuppressed, uncolonized individuals who come in contact with colonized persons.

It is known that animals carry *Pneumocystis* asymptomatically, and that colonized, immunocompetent animals can transmit the infection to co-housed immunocompetent animals, but the degree to which colonization exists in healthy humans or fosters pathogen transmission is unknown [35]. Healthy, nonimmunosuppressed adults have generally not been found to harbor *P. jirovecii* DNA in induced sputum samples or autopsy studies [29, 30, 36, 37], suggesting that colonization may be rare in this population. However, an immunosuppressed state may place the body at increased risk for colonization. Between 10 and 69% of HIV-infected persons and 7–19% of patients with underly-

ing chronic illnesses or various respiratory disorders are colonized with *P. jirovecii* [38, 39], and the frequency of colonization is known to increase with declining CD4 counts [36].

If and how colonization affects the risk of eventual disease development remains unknown. One study showed that, of eight HIV-positive, highly immunosuppressed but asymptomatic persons colonized with *Pneumocystis* species (as detected by polymerase chain reaction [PCR]), six developed infection 6 months to 1 year after their positive PCR result [28], suggesting that colonization may increase the risk of subsequent disease. Still, not all colonized persons progress to disease. Several studies have described asymptomatic, immunosuppressed patients who are PCR-positive for *P. jirovecii* but who did not progress to PCP when observed for several months [27, 36, 40, 41]. Asymptomatic carriage after disease resolution has also been described [27, 42]. A few studies have suggested that colonization may be distinguished from true infection using quantitative PCR methods with diagnostic cutoff values [27, 42], but these methods have not been standardized.

If colonization does increase the risk of disease development, screening strategies may be warranted so that prophylaxis might begin at an earlier stage of HIV disease than is currently recommended. At least one study has shown prophylaxis to be effective at containing PCP infection in colonized patients, although prophylaxis did not prevent or reverse colonization itself [27].

Prophylaxis and Treatment

Both prophylaxis and treatment for *P. jirovecii* use trimethoprim-sulfamethoxazole (TMX). Dapsone, pentamidine, clindamycin/primaquine, and atovaquone are also prophylaxis and treatment options [43•], but TMX is considered superior because of its increased efficacy, lower cost, and protection against other infections. Side effects of TMX are generally mild but can include fever, rash, and neutropenia.

PCP prophylaxis is recommended for all HIV-infected patients with CD4 T-cell counts below 350/μL [44]; however, if the patient is on highly active antiretroviral therapy (HAART), PCP prophylaxis can be stopped once the CD4 T-cell counts rise above 350/μL for 6 months or more on HAART [45, 46]. The World Health Organization recommends that infants born to HIV-infected mothers be started on prophylaxis at 4 to 6 weeks of age until 1 year; those determined to be HIV-uninfected can discontinue therapy unless breastfeeding [44]. Children with a history of PCP, however, should maintain lifelong prophylaxis [47]. For allogeneic hematopoietic stem cell transplant (HSCT) patients, prophylaxis is recommended throughout all periods of immune compromise after engraftment, or before engraftment if it is delayed [48], although myelosuppression resulting from PCP prophylaxis can itself delay engraftment. For autologous HSCT patients, prophylaxis is recommended only under certain circumstances [48].

Drug Resistance

Much controversy surrounds the field of *P. jirovecii* infection and drug resistance. Because cotrimoxazole is perceived to have additional benefits beyond prophylaxis or treatment of PCP [49, 50], it is often prescribed broadly for persons with HIV and pneumonia. Duration of sulfa exposure is linked to mutations in the dihydropteroate synthase (DHPS) locus of *Pneumocystis*, which encodes an enzyme targeted by sulfa drugs [51]. Although DHPS mutations have been linked to drug failure [52] and reduced patient survival in at least one study [51], other studies have failed to demonstrate an association [52–54]. It is possible that these mutations represent a "halfway point" in the evolution of resistance in this pathogen. Again, progress in this realm is hindered by an inability to grow the organism in culture. However, history has repeatedly shown that overtreatment engenders drug resistance among any number of pathogens, so increasing signs of molecular resistance among infecting PCP strains [34] should warrant attention from the global community and possibly preemptive action to ensure the prudent use of cotrimoxazole.

Diagnosis

Currently, there are no methods available to culture *P. jirovecii*. Because of the difficulty in growing the organism in culture and the resultant lack of a diagnostic "gold standard," standardization of diagnostic methods has been challenging. Perhaps because of the lack of guidance surrounding "best practices" for diagnosis, the absence of clear cost-effectiveness data regarding diagnostic options, and the controversy over the magnitude of the drug resistance problem, PCP is often diagnosed—and treated—empirically.

PCP diagnosis is a two-step procedure involving specimen collection and subsequent laboratory-based diagnosis. Specimens can include oral washes, expectorated or induced sputum, tracheal secretions, broncho-alveolar lavage (BAL), or native or paraffin-fixed lung biopsy specimens from patients suspected of having an infection. The highest sensitivities are achieved using BAL or biopsy [41] specimens, with a sensitivity of 89–98% [38], using direct observation as the laboratory-based diagnostic method. The technical complexity and invasiveness of obtaining these specimens, however, makes them difficult to collect in many low-resource areas and in some extremely sick

patients; as a result, sputum (induced or expectorated) is often used for diagnosis, which may lead to false negative results.

Multiple laboratory diagnostic methods, ranging from direct visualization to PCR-based methods, can be employed on all specimen types. Visualization of the organism can be performed using immunofluorescence microscopy (IF), cyst wall stains (Grocott, Toluidine Blue O (TBO), and calcofluor white (CW)), or trophozoite stains (Wright-Giemsa, Diff-Quick, and Papanicolaou). However, subjective interpretation of results makes these methods potentially nonspecific, and sensitivity depends on the type of specimen and the skill of the technician examining the sample.

Single-round PCR, nested PCR, and quantitative real-time (RT)-PCR [32, 42, 55, 56•] can also be used to detect *Pneumocystis* DNA in specimens. However, though PCR is acknowledged to be the most sensitive method available for detecting PCP [32, 57], concerns exist about its specificity, as it may detect colonization in the absence of clinical infection. In a study involving 22 asymptomatic patients who were positive for *Pneumocystis* DNA by single-round PCR, but in whom organisms were not detected in initial BAL specimens, several were found to have detectable organisms in a subsequent BAL specimen or to respond positively to empiric PCP therapy, suggesting that they were infected rather than colonized. In this study, when these seven patients were counted as "true positives," single-round PCR was 100% sensitive and 81% specific [57]. Quantitative RT-PCR methods may help distinguish colonized patients from infected patients [27, 42].

The use of PCR on oral wash specimens shows particular promise as a noninvasive diagnostic method. One study showed nested PCR to be insufficiently sensitive in its detection of *Pneumocystis* DNA in oral wash specimens (compared with induced sputum specimens) [58], but another study found quantitative, touch-down PCR to be 89% sensitive and 94% specific for detecting both active infection and colonization using oral washes [55]. The authors found the same method to be 88% sensitive and 85% specific for detecting PCP in oral washes in a prospective study, although patient treatment before specimen collection decreased the method's sensitivity [59]. PCP-specific primers did not amplify DNA in oral wash specimens from *Escherichia coli*, *Streptococcus pneumoniae*, viridans group streptococci, *Klebsiella pneumoniae*, *Haemophilus influenzae*, cytomegalovirus, herpes simplex virus, Epstein-Barr virus, varicella zoster virus, *Aspergillus fumigatus*, *Candida albicans*, *Cryptococcus neoformans*, *Coccidioides immitis*, *Mycobacterium tuberculosis*, *Mycobacterium avium*, or *Histoplasma capsulatum* [57], showing that the primers are organism-specific.

Global Burden and Epidemiology

Global estimates of PCP prevalence among HIV-infected patients vary widely by country and study. Differences in methods of patient recruitment, prophylaxis, and diagnosis make comparisons between studies difficult at best.

Industrialized Countries

PCP still occurs in industrialized countries among HIV-infected infants (in whom PCP is not related to absolute $CD4^+$ count in the same manner as in adults), HIV-infected children, and other immunosuppressed persons. Children were most affected by PCP at the start of the HIV pandemic, and although HAART and prenatal testing have decreased vertical transmission and immunosuppression, HAART is still relatively newer for children than for adults, and the full effect of therapy in decreasing PCP among children is probably not yet realized in the United States [3]. Improved maternal HIV screening (and appropriate infant PCP prophylaxis) is likely the only factor that could reduce cases of pediatric PCP in the United States.

Several studies track PCP in HIV-infected adults in industrialized countries, including the US Centers for Disease Control (CDC) Adult and Adolescent Spectrum of HIV Disease (AASHD) project, the Multicenter AIDS Cohort Study, and the EuroSIDA study. In all industrialized countries, PCP has declined substantially since the inception of HAART therapy [7–9, 60]. Among adult patients in the United States not receiving or responding to HAART, however, PCP is still the most common AIDS-defining opportunistic infection [3]. Of HIV/AIDS patients discharged from the hospital between 1996 and 2005, 9% were diagnosed with PCP [61]. In the AASHD project, nearly 10% of 1,073 PCP patients between 1999 and 2001 had not received appropriate prophylaxis before their infection [3]. A recent study of HIV-infected patients treated for PCP at a San Francisco hospital showed a mortality rate among these patients of 10.3% [62]. Older age, recent intravenous drug use, elevated bilirubin, depressed albumin, and elevated alveolar-arterial oxygen gradient were significant predictors of mortality.

Cases and outbreaks not associated with HIV infection are generally limited to probable human-to-human transmission incidents among solid organ transplant recipients [21•, 63–66], among whom the incidence of PCP has been estimated at approximately 1% [67]. Use of the immunosuppressive drug sirolimus among these patients was associated with increased risk of PCP, and a PCP diagnosis was associated with graft loss and death [68].

Africa

Even after the inception of the HIV pandemic, PCP was considered a rare disease among adults in sub-Saharan Africa [69–74]. This finding may be due to decreased virulence of African strains of *Pneumocystis* species, genetic resistance to the organism, or a simple infrequent presence of the organism in Africa; however, the latter seems unlikely, as HIV-infected African children have historically had high rates of PCP [3]. Because of the extremely high rates of TB in Africa, PCP in African adults is frequently misdiagnosed as smear-negative TB [75–77].

In the past 10–15 years, however, increases in adult PCP diagnoses have been noted in many parts of Africa [32, 78–82]. Changes in diagnostic methods have been suggested as a possible cause for this apparent increase. However, when early and more recent studies using comparable diagnostic methods are compared, rates of PCP among African adults during the late 1980s were still lower than they are today (reviewed by Fisk et al. [83]), suggesting that the observed increase in disease may not be explained by diagnostic changes. Regardless of the reason for the increase, several studies have suggested that PCP may no longer be rare among HIV-infected adults in some areas of Africa [77, 78, 83]. It is possible that significant interregional variation in PCP in Africa exists, and that it cannot be considered in a uniform manner.

During the 1980s and 1990s, HIV-infected children appeared to have much higher rates of PCP than HIV-infected adults in Africa [3]. Autopsy studies of pediatric HIV patients in various parts of Africa between 1992 and 2000 showed PCP rates between 16% and 29% [3], though these figures may be biased, as they represent terminal disease. Studies of hospitalized pediatric HIV patients in South Africa from 1999 to 2001 showed a PCP prevalence of 44–49%; one 1998 study found a PCP prevalence of 10%, but the authors were unable to follow negative sputum exams with bronchoscopy, suggesting that this number could be a serious underestimate [3]. A recent study of 202 HIV-infected and HIV-uninfected children with pneumonia admitted to a South African hospital found that 21% had PCP; of these, 77% were HIV-infected, and most of the others were HIV-exposed and/or malnourished. Despite the availability of free HAART and PCP prophylaxis, only 18% of the patients were receiving prophylaxis [84••]. Mortality rates among HIV-infected African children with PCP are high (20–80%), perhaps because of treatment delays until PCP is at an advanced stage, or a lack of treatment [83].

Asia

In Asia (particularly southeast Asia), PCP has been reported in both children and adults. In Thailand, the prevalence of disease has been reported between 27 and 40% among HIV-infected patients seen at a university hospital clinic [85, 86]. Cases of PCP increased in Thailand between 1992 and 2003, from fewer than 100 cases per year to more than 6,000 cases per year [87]. A study tracking AIDS-defining illnesses in Thailand over time found that about 20% of AIDS patients' AIDS-defining illnesses were PCP between 1994 and 1998 [88]. Fever of unknown origin among HIV-infected adults was found to be due to PCP in 13% of cases [89•]. Among pediatric AIDS patients in Thailand, the prevalence of PCP has been shown to be between 40% and 66% [85, 90]. In Malaysia, PCP was found to be the second most common AIDS-defining illness (after TB) among 419 HIV/AIDS patients seen at a Kuala Lumpur hospital between 1994 and 2001 [91].

PCP is also of growing concern in Cambodia. In 2000, it was estimated that HIV prevalence among persons 15–49 years of age was nearly 3%, the highest among Southeast Asian countries [92, 93]. A chart review of 351 patients with HIV admitted to the hospital for various reasons showed that 8% had PCP [93]. A later study of 100 patients with advanced HIV disease showed that non-induced sputum from 10% of patients contained *Pneumocystis* organisms [94]. In a third study, among 193 HIV-infected patients in Cambodia with AFB-smear–negative pneumonia, 44% were shown to have PCP [92].

Central and South America

PCP was the second most common complication, at 22%, among HIV-infected patients enrolled in a surveillance program in Brazil for AIDS-associated illnesses, carried out between 2001 and 2004 [95]. As in Africa, tuberculosis is the primary AIDS-defining disease in this population [96]. In Argentina, PCP was identified during chart review as a cause of hospitalization in 6–9% of AIDS patients between 1995 and 2002 [97]. Among 132 HIV-infected infants and children in Argentina, PCP was the second most common AIDS-defining disease (35%) and was particularly lethal among infants [98]. The same study showed that PCP prophylaxis, prescribed beginning in 1996, decreased mortality in this population.

Conclusions

In spite of its historic and ongoing role as a primary AIDS-defining disease, critical knowledge gaps still exist about *P. jirovecii*. Because of the inability to culture the organism in the laboratory, the search for an animal or environmental reservoir is challenging, and studies of pathogenesis in humans, the life cycle, disease transmission, and genotypic differences between strains are hampered. Existing diag-

nostic methods are suboptimal, and their cost-effectiveness is poorly defined, leading to empiric diagnoses in many parts of the world, along with poor economic decision-making. Because of the difficulty in standardizing diagnostic methods, the global burden of disease is not well understood, and there are few recent, broad-range data on the prevalence of infection worldwide. In addition, questions remain about colonization: What factors influence colonization? Is colonization a prerequisite to infection, and if so, should colonized patients be treated? Are colonized patients a potential source of infections for their uninfected contacts? Drug resistance and its relationship to DHPS mutations and duration of prophylaxis are not well understood. Questions about differences in disease epidemiology globally—such as whether the incidence of PCP is truly increasing among African adults—remain unanswered. Without these data, appropriate control measures cannot be developed. Well-designed studies with a global reach are needed to define the burden of disease locally and globally, and to develop recommendations for control and prevention of disease.

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- •• Of major importance

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