Epidemiology of *Pneumocystis carinii* Infection: Application of Molecular Genetic Methods

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_Pneumocystis carinii* causes a life threatening, diffuse, extracellular infection of the pulmonary alveoli in 20–70% of immunosuppressed subjects with AIDS, organ transplantation or malignancy. Although animal model experiments favour airborne transmission of infection, failure to culture *P. carinii* has impeded efforts to clarify its epidemiology. A high prevalence of positive serology to *P. carinii* in normal individuals has suggested, by analogy with tuberculosis, that opportunistic pneumonia in the immunosuppressed individual results from recrudescence of a dormant pulmonary focus of organisms, the legacy of childhood infection. We have cloned a series of mitochondrial genes from *P. carinii* as a basis for i) conducting comparative DNA sequence studies and ii) providing phylogenetic information for the development of a highly specific and sensitive DNA amplification technique for recognizing the parasite in different samples. We have not found carriage of *P. carinii* in normal lungs and have shown that *P. carinii* organisms infecting different mammalian hosts are genetically distinct. Comparative DNA studies based on DNA amplification products using *P. carinii* specific primers at reduced annealing temperatures in the polymerase chain reaction, suggest that *P. carinii* is most closely related to the ustomycetous red yeast fungi. These organisms are found freely in the environment and produce widely disseminated air-borne spores. These data suggest that opportunistic *Pneumocystis* pneumonia results from fresh infection, acquired from an environmental source.

**Keywords:** *Pneumocystis carinii*, Molecular genetics, AIDS, Cancer chemotherapy, Transplantation.


_Pneumocystis carinii* is a major cause of opportunistic pneumonia in patients with T-lymphocyte immune deficiency, now principally those on transplantation and cancer chemotherapy programmes and those with AIDS. *Pneumocystis* causes a diffuse pneumonia, characterized by bilateral radiographic change and hypoxaemia, which is confined to the extracellular compartment.
of the alveolar space. Untreated it is fatal but prompt treatment with cotrimoxazole or pentamidine can rescue 80% of patients.

The organism, first recognized by Chagas and Carini, cannot be effectively cultured in vitro and this has seriously impeded progress in understanding the basic biology of this opportunistic parasite, its taxonomy and epidemiology. Until fairly recently, both disease and epidemiological studies on infection depended on recognition of the parasite by microscopy, either the cyst form after silver staining or the trophozoite form, after Giemsa staining.

**Epidemiology of Disease and Infection**

Pneumocystis was first demonstrated to be a cause of human disease in malnourished, institutionalized infants in Eastern Europe in the middle decades of this century. The mode of transmission was unclear though it was considered by some that isolation of cases might be helpful in preventing spread.

Subsequent epidemiological studies, conducted by microscopy and silver staining on post mortem lung samples from children and adults, with and without immune deficiency, suggested the possibility of low grade carriage of the organism in the lung, even of healthy individuals.

Serological surveys, using pneumocystis antigen purified from infected lungs, suggested regular infection of healthy children with a cumulative prevalence of 80% by the age of 10 years. These observations led to the conclusion that opportunistic pneumocystis pneumonia in the immunosuppressed, by analogy with tuberculosis, was due to recrudescence of a dormant focus of organisms in the lung which was the residue of early infection in childhood.

Attempts to understand the mode of transmission were only approachable by animal model experiments. In these, Hughes et al. showed that rats, immunosuppressed by dexamethasone in their drinking water, acquired pneumocystis pneumonia when simply exposed to ambient air; this suggested air-borne transmission of disease.

**Molecular Genetic Approaches**

More recently, clones of pneumocystis-specific DNA have been isolated from infected lung and have laid the foundation for the development of a highly specific and sensitive technique for identifying pneumocystis in clinical and epidemiological surveys using the method of DNA amplification by the polymerase chain reaction. This method allows for the identification of the parasite in any particular sample by *in vitro*, chemical amplification of a specific DNA sequence of pneumocystis and the identification of this specific DNA by its length characteristics, using electrophoresis, and by its hybridizing properties, using DNA probing.

**Diagnostics**

Using DNA sequence for mitochondrial ribosomal RNA, Wakefield *et al.* developed a DNA amplification method that was used as a diagnostic technique on bronchoscopic lavage and induced sputum samples from patients with AIDS and unexplained pneumonia. In both samples, the DNA amplification method produced large, diagnostic quantities of pneumocystis-specific DNA in over 95% of cases, with a diagnostic specificity for pneumocystis pneumonia of 98%. Smaller quantities of pneumocystis-specific DNA were amplified in a quarter of AIDS subjects and this was interpreted as colonization; the DNA amplification method can recognize as few as one or two organisms in a PCR reaction.

**Latent infection**

Because of the specificity and sensitivity of the *P. carinii* DNA amplification method, the studies were extended to epidemiological surveys and in particular to the screening of post mortem lungs from non-immunosuppressed individuals to search for latent pneumocystis infection. In a series of tests, no organism was identifiable by PCR and this result accorded with studies using monoclonal antibodies to screen healthy lungs. Both sets of results suggest that the earlier findings (identifying ‘cysts’ by silver staining in normal lungs) were not specific and therefore that regular carriage of pneumocystis does not occur in the healthy lung. Thus opportunistic pneumonia in the immunosuppressed is unlikely to be due to recrudescence of a dormant focus of organisms; this implies that such pneumonia is acquired as a fresh infection in the immunosuppressed and the source of this infection is of especial interest.

**Infection in other mammals**

Though pneumocystis has been recognized as a cause of pulmonary infection in other mammals, it has been unclear whether transmission from that source might be a cause of human disease. Recently, comparative sequencing of DNA, obtained by PCR from pneumocystis isolates from different hosts, has shown that there are host-specific strains, characterized by their DNA sequence,
which are unlikely readily to cause disease in a variety of hosts.

**Taxonomy**

Studies on the taxonomy of the organism are also relevant to the epidemiology of infection. A number of studies have now been published on comparative DNA sequencing of pneumocystis in relation to a range of other organisms.\(^\text{19,20}\) It is clear from these that pneumocystis is related to the fungi and not to the protozoans, as otherwise thought.\(^\text{21}\) Significant similarity to the fungi is observed at a number of DNA loci.\(^\text{20}\)

In further studies on DNA encoding mitochondrial ribosomal RNA, Wakefield, et al. have shown, in particular, that pneumocystis is closely aligned to the ustomycetous (or basidiomycetous) red yeast fungi.\(^\text{22}\) These fungi are of especial note since they disseminate their spores by a ballistospore mechanism over long ranges and in high densities through ambient air from their environmental sources which include a range of vegetation and water. The affinity of pneumocystis with these fungi suggests that it may also disseminate large numbers of spores from an, as yet, unidentified environmental source and which could be the origin of fresh opportunistic infection in the immunosuppressed. This mechanism is reminiscent of other fungal pulmonary diseases of man, including cryptococcus, histoplasmosis and coccidioidomycosis.

The likelihood of infection from an environmental source does not exclude the possibility of additional, horizontal transmission between immunosuppressed individuals.\(^\text{23}\) Further studies aiming to track pneumocystis infection in the AIDS population, and utilizing DNA polymorphism to characterize pneumocystis strains, may be valuable in this respect.

**Conclusion**

Molecular genetic techniques are proving valuable in effective diagnosis of opportunistic pneumocystis pneumonia but also in investigating the organism’s basic biology and the epidemiology of infection.

**References**


