**Genetic characterization of Pneumocystis jirovecii in patients dying of AIDS, IPK, 1995-2008**

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**ABSTRACT**

*Pneumocystis jirovecii* is a frequent opportunistic pathogen in HIV/AIDS. The inability to culture this species prevents from acquiring deeper knowledge about its biology, drug susceptibility as well as the epidemiology of the disease that it produces. Pneumocystosis diagnosis in Cuba is suspected, based on the clinical and radiological findings which often lead to misdiagnosis since other microorganisms produce symptoms, signs and radiological patterns similar to this infection. Currently, the status on drug resistance is unknown and there are no means to evaluate the resistance to the recommended drugs. On the other hand, no studies on the distribution of *P. jirovecii* of genotypes in the country have been described. The results of the present study allows: a) to describe for the first time in the literature a new genotype of mitochondrial small RNA ribosomal subunit (160A/196T), b) to suggest a narrow circulation of the pathogen in Cuba; and c) to assume a very low prevalence of sulfamide resistance linked to mutations of *P. jirovecii* in our country. The results obtained constitute highly valuable knowledge of pneumocystosis in Cuba, as well as worldwide since they provide new information that can be used to a better disease management.

**Keywords:** pneumocystis, genetic characterization, resistance

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**Introduction**

*Pneumocystis jirovecii pneumonia* (PcP) is considered one of the most common opportunistic diseases in patients with human immunodeficiency virus (HIV). Despite the use of highly active antiretroviral therapy and chemophrophylaxis for its control it continues to cause a negative impact on health of such patients in many countries [1, 2]. Today, PcP is still the most prevalent opportunistic infection among acquired-immunodeficiency syndrome (AIDS) patients, although it is becoming more frequent among subjects with non-AIDS-related immunosuppression. The lack of a microbiological culture method for this species constitutes a major obstacle for increasing our knowledge about its biology and susceptibility to drugs as well as the epidemiology of the disease [1]. For the above mentioned reasons the molecular tools for the detection and characterization of this atypical fungus come to hand. Among the molecular targets used for the study of this microorganism there are genes encoding the mitochondrial large and small RNA ribosomal subunits and the gene encoding for dihydropteroate synthase, where mutations inducing drugs resistance have been described [1].

In Cuba, only four published scientific articles have addressed this subject. The first case of PcP in the country was reported in 1969 by Rodriguez-Vigil [3]. Later, Razón Behar et al. [4] described the infection in seven malnourished children, thereby performing the first contributions to the clinic and epidemiology of the disease in the country. On the other hand, Menendez-Capote and Millan-Marcelo [5] found a 45% rate of the disease among 40 HIV/AIDS patients admitted at the Institute of Tropical Medicine Pedro Kouri (IPK). These diagnoses were based only on clinical symptoms and radiographic patterns of the patients examined. Finally, Arteaga et al. [6] found...
32% of PCP in 211 post mortem studies performed to AIDS patients with severe immunosuppression in the 1986-1998 period, at the IPK.

Basically, the PCP diagnosis is performed by the observation of any life stage of the pathogen under the microscope in respiratory samples, yielding the best results those obtained by bronchoscopy. However, this is an invasive procedure for the patient and in many countries is not performed as a routine procedure. Currently, few studies addressing the characterization of P. jirovecii in paraffin embedded tissues have been reported in the scientific literature [7].

The diagnosis of PCP in Cuba is essentially based on the clinical and radiological findings, which can lead to errors since there are etiological agents that produce similar patterns to those described in infection with P. jirovecii. Moreover, in Cuba, the current status on P. jirovecii resistance to the drugs is unknown and there is no method to evaluate it. This may lead to a major therapeutic problem when the patients with clinically diagnosed PCP do not improve when treat this infection. Since the procedure only takes about 24-48 hours, patients will receive the specific treatment as soon as possible.

Conclusions
This study provides new scientific information on the biology, genetics, epidemiology and sulfamide drug resistance of P. jirovecii for Cuba. The genetic information resulted in an interesting piece of information for the scientific community of the world. It also shows the usefulness of paraffin embedded tissues in molecular epidemiology studies, which could be taken into consideration by the scientific community. Finally, this work plays an important role in the diagnosis and characterization of pathogens found in samples of HIV/AIDS patients in Cuba, which until now, lacked a reliable, accurate and fast diagnostic method. At the same time, represents a direct approach to assess the possible resistance against the drug used to treat this infection.

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Results and relevance of the study
The results of this study represent the first genetic characterization of P. jirovecii in Cuban samples of any kind and origin. Besides, our study is considered the first report describing the existence of a new genotype of P. jirovecii mitochondrial small subunit ribosomal RNA gene in the world, indicated vs. the sequence deposited in GenBank with the accession number HQ228547 (Figure).

The obtained results also have other novel aspects of scientific importance to consider. The first genetic characterization of multilocus P. jirovecii in paraffin tissues is reported. Similarly, it is the only report in the international literature describing the molecular epidemiology of P. jirovecii in a 14-year study in a country.

The information provided by this research allows hypothesizing on a narrow genetic variability of this pathogen in a country/island, in contrast to the situation described for the continents. Moreover, this is the first report on the lack of resistance to sulfonamides in P. jirovecii samples in Cuba [8]. Finally, the authors consider that this study has an important social impact because technical capacities are now available at the IPK hospital to provide specific diagnosis for this fatal disease. The procedure only takes about 24-48 hours, patients will receive the specific treatment as soon as possible.

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This study provides new scientific information on the biology, genetics, epidemiology and sulfamide drug resistance of P. jirovecii for Cuba. The genetic information resulted in an interesting piece of information for the scientific community of the world. It also shows the usefulness of paraffin embedded tissues in molecular epidemiology studies, which could be taken into consideration by the scientific community. Finally, this work plays an important role in the diagnosis and characterization of pathogens found in samples of HIV/AIDS patients in Cuba, which until now, lacked a reliable, accurate and fast diagnostic method. At the same time, represents a direct approach to assess the possible resistance against the drug used to treat this infection.

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