

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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RESUMEN

Caracterización genética de *Pneumocystis jirovecii* en fallecidos por sida, IPK, 1995-2008. *Pneumocystis jirovecii* es un agente patógeno oportunista que frecuentemente afecta a los enfermos con el VIH/sida. La imposibilidad de su cultivo limita el conocimiento profundo de su biología, susceptibilidad a los fármacos, y la epidemiología de la neumocistosis que provoca. En Cuba, se diagnostica mediante la información clínico-radiológica del paciente; pero esta puede ser inespecífica y acarrear errores, pues otros microorganismos provocan síntomas, signos y patrones radiológicos similares a los de esa infección. A su vez, no poder evaluar la resistencia a los medicamentos es un obstáculo en el tratamiento completo al paciente con VIH/sida. Ningún estudio describe la distribución de los genotipos de *P. jirovecii* en Cuba. Los resultados que se presentan a) revelan un nuevo genotipo (160A/196T) del gen que codifica para la subunidad menor del ARNr, b) sugieren una circulación restringida del agente patógeno en Cuba, c) e indican que la prevalencia de *P. jirovecii* con mutaciones vinculadas con su resistencia a sulfamidas es muy baja en nuestro medio. Tales resultados son de extraordinario valor no solo para el conocimiento de la neumocistosis en Cuba y en el mundo, sino también para el tratamiento integral a pacientes con VIH/sida.

Palabras clave: pneumocystis, caracterización genética, resistencia

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 chemoprophylaxis 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 Rodríguez-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 Kourí (IPK). These diagnoses were based only on clinical symptoms and radiographic patterns of the patients examined. Finally, Arteaga *et al.* [6] found

1. Calderon EJ, Gutierrez-Rivero S, Durand-Joly I, Dei-Cas E. *Pneumocystis* infection in humans: diagnosis and treatment. *Expert Rev Anti Infect Ther.* 2010; 8(6):683-701.

2. de Armas Rodriguez Y, Wissmann G, Muller AL, Pederiva MA, Brum MC, Brackmann RL, *et al.* *Pneumocystis jirovecii* pneumonia in developing countries. *Parasite.* 2011;18(3):219-28.

3. Rodríguez-Vigil E. Neumonía intersticial por *Pneumocystis carinii*. *Rev Cubana Pediatr.* 1969;41:317.

4. Razón Behar R, Cubero Menéndez O, Vázquez Ríos B, Chao Barreiro A, Gala Valiente M, Cubeñas Chala YD. *Pneumocystis carinii* pneumonia. *Rev Cubana Med Trop.* 1977;29(3):103-14.

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 the adequate therapeutic regime is imposed. Significantly, the distributions of *P. jirovecii* genotypes in the country have never been studied. For these reasons, we decided to undertake this study on this fascinating pathogen. This research was granted the 2011 Award of the Cuban National Academy of Sciences.

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

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1 GAAGCATGTT GTTTAATTCG ATAACCCACG ATAAATCTTA CCACTTCTTG CATATTTTCC
61 TATTCCGAAT TTACAGGTGT TGCATGGCTG TCTTTAGTTC GTGTTGTGAA ATGTTAGGTT
121 TATTCCGATA ACGAACGTAA ACCTTGTCTT TAATTATTTA AAGGAAATGT CTATCGATAT
181 TATAGATGAA TTAGGTAGAA GACAAGTCCT CATGACCCTT ATGAAGTGGG CTACAGACGT
241 GCTGCAAAT TTTCTACAAT GGGATGCAAT GATGGAAGTC GGAGCTAATC CCCTAAAAGA
301 TTGTTTAGTC CCGATAAGTG CCTGGAAGTC GGCTCTTT

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Figure. Partial sequence of the *P. jirovecii* mitochondrial small subunit ribosomal RNA gene (GenBank accession number HQ228547), with nucleotides identifying the new genotype (160T→A/196A→T) highlighted.

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. 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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5. Menendez Capote R, Millan Marcelo JC. [Infections and other opportunistic processes in a group of Cuban stage-IV HIV patients]. *Rev Cubana Med Trop.* 1992; 44(1):47-9.

6. Arteaga Hernandez E, Capo de Paz V, Perez Fernandez-Teran ML. Micosis oportunistas invasivas en el sida. Un estudio de 211 autopsias. *Rev Iberoam Micol.* 1998;15(1):33-5.

7. de Armas Y, Capó V, López Fuentes L. Detección molecular de *Pneumocystis jirovecii* en tejido parafinado de fallecidos por VIH/sida. *Rev Cubana Med Trop.* 2008 [cited 2012 May 17];60(3):[about 7 p.]. Available from: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0375-07602008000300009&lng=en&nrm=iso&tlng=es.

8. de Armas Y, Friaza V, Capo V, Durand-Joly I, Govin A, de la Horra C, *et al.* Low genetic diversity of *Pneumocystis jirovecii* among Cuban population based on two-locus mitochondrial typing. *Med Mycol.* 2012;50(4):417-20.