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AIDS-related *Pneumocystis jirovecii* genotypes in French Guiana

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This study is dedicated to the memory of Eduardo Dei-Cas who shared his enthusiasm for *Pneumocystis* with us.

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ABSTRACT

The study described *Pneumocystis jirovecii* (*P. jirovecii*) multilocus typing in seven AIDS patients living in French Guiana (Cayenne Hospital) and seven immunosuppressed patients living in Brest, metropolitan France (Brest Hospital). Archival *P. jirovecii* specimens were examined at the dihydropteroate synthase (DHPS) locus using a PCR-RFLP technique, the internal transcribed spacer (ITS) 1 and ITS 2 and the mitochondrial large subunit rRNA (mtLSUrRNA) gene using PCR and sequencing. Analysis of typing results were combined with an analysis of the literature on *P. jirovecii* mtLSUrRNA types and ITS haplotypes.

A wild DHPS type was identified in six Guianese patients and in seven patients from metropolitan France whereas a DHPS mutant was infected in the remaining Guianese patient. Typing of the two other loci pointed out a high diversity of ITS haplotypes and an average diversity of mtLSUrRNA types in French Guiana with a partial commonality of these haplotypes and types described in metropolitan France and around the world. Combining DHPS, ITS and mtLSU types, 12 different multilocus genotypes (MLGs) were identified, 4 MLGs in Guianese patients and 8 MLGs in Brest patients. MLG analysis allows to discriminate patients in 2 groups according to their geographical origin. Indeed, none of the MLGs identified in the Guianese patients were found in the Brest patients and none of the MLGs identified in the Brest patients were found in the Guianese patients.

These results show that in French Guiana (i) PCP involving DHPS mutants occur, (ii) there is a diversity of ITS and mtLSUrRNA types and (iii) although partial type commonality in this territory and metropolitan France can be observed, MLG analysis suggests that *P. jirovecii* organisms from French Guiana may present specific characteristics.

1. Introduction

The transmissible fungus *Pneumocystis jirovecii* (*P. jirovecii*) is the causative agent of severe pneumonia [*Pneumocystis* pneumonia (PCP)] in immunosuppressed patients (Walzer and Cushion, 2005). PCP remains the most frequent AIDS-defining illness in human immunodeficiency virus (HIV)-infected patients in developed countries including metropolitan France (Cazein et al., 2013). In contrast, in French Guiana, disseminated histoplasmosis, esophageal candidiasis, cerebral toxoplasmosis and tuberculosis are the four most frequent AIDS-defining illnesses, whereas PCP occupies the fifth position (Nacher et al., 2011). HIV/AIDS is a major public health issue in this overseas French territory in which AIDS incidence is 216 per 1,000,000 inhabitants vs. 21 in France (Cazein et al., 2013) and HIV incidence is 914 per 1,000,000 in French Guiana vs. 170 per 1,000,000 in France (Cazein et al., 2013). These data point out specific geographical features of HIV infection and its related opportunistic microorganisms, specifically

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P. jirovecii. In this context, the fact that *P. jirovecii* organisms may differ from those in Metropolitan France can be hypothesized. At present, it is unknown if *P. jirovecii* organisms in French Guiana present specific characteristics. Indeed, data concerning *P. jirovecii* genotypes from French Guiana are still scarce, while French *P. jirovecii* organisms that have been characterized by genotyping are mostly those obtained from metropolitan France (Latouche et al., 1997; Le Gal et al., 2013; Nevez et al., 2003; Totet et al., 2003). As far as we know, the typing of *P. jirovecii* from Guianese patients has been reported in only one instance (Le Gal et al., 2011). In this case, published elsewhere, we described the preliminary results of unilocus typing of one *P. jirovecii* specimen from an AIDS patient (Le Gal et al., 2011). The present study reports the multilocus typing of *P. jirovecii* organisms at the locus of the dihydropteroate synthase (DHPS), the enzymatic target of the sulfonamides, the internal transcribed spacer (ITS) 1 and 2 locus, and the mitochondrial large subunit rRNA (mtLSUrRNA) gene, from AIDS patients with PCP living in this overseas French territory. *P. jirovecii* types identified in this patient population were compared with those identified at the same loci in immunosuppressed patients living in metropolitan France, and with those reported worldwide, through an analysis of the literature on *P. jirovecii* ITS haplotypes and mtLSUrRNA types.

2. Materials and methods

2.1. P. jirovecii specimens and patients

Seven *P. jirovecii* specimens from seven patients [median age 33 years (range, 30–57); sex ratio male/female 1/6] who developed PCP and who were monitored in the Andrée Rosemon Hospital, Cayenne, French Guiana, were retrospectively studied. All patients were HIV-infected. The first patient was monitored in 2002 [previously described in part in (Le Gal et al., 2011)] whereas the six other patients were diagnosed with PCP over a one-year period from November 2011 through October 2012. Clinical and biological data of patients are shown in Table 1.

Seven *P. jirovecii* specimens from seven other patients [median age 42 years (range, 26–73 years); sex ratio male/female 4/3] who had developed PCP and who were monitored in the Brest University Hospital, Brest, France between October 2007 and November 2009, were also retrospectively studied. Five patients were immunosuppressed due to organ transplantation or cancer. The two remaining patients were HIV-infected. They represented a control group providing data on genotypes of *P. jirovecii* organisms from the Brest region in metropolitan France. Clinical and biological data of patients are shown in Table 1.

Patients of the two groups had undergone a bronchoalveolar lavage (BAL) procedure to investigate pulmonary symptoms or fever. *P. jirovecii* had initially been detected in BAL specimens by microscopy using Wright-Giemsa stain and an indirect immunofluorescence assay (MonofluoKit *Pneumocystis*, Bio-Rad, Marnes-La-Coquette, France). BAL sediments from the two patient groups were stored at −80 °C until typing.

2.2. Multilocus typing

The DNA extraction of the BAL specimens was performed using QIAamp DNA MiniKit (Qiagen, Courtaboeuf, France). The typing at the DHPS locus was performed using a PCR-RFLP assay as we reported elsewhere (Le Gal et al., 2012). First, the DHPS sequences were amplified using a nested-PCR assay. Second, the RFLP assay was performed with two restriction enzymes, *Acc*I and *Hae*III, according to the manufacturer's recommendations (New England Biolabs®, Ipswich, MA, USA). PCR product digestion makes it possible to detect the two main non-synonymous mutations at nucleotide positions 165 and 171. A wild type ($A^{165}C^{171}$) has an A residue at nucleotide position 165 and a C residue at position

Table 1
Characteristics of French Guianese patients and French metropolitan patients who developed *Pneumocystis* pneumonia and for whom *Pneumocystis jirovecii* specimens were genotyped.

Patient code[a]	Gender[b]	Age	Underlying conditions	Blood CD4$^+$ T cell count, 10^6/liter	Prior sulfonamide treatment[c]	Date of BAL[d] (mo-day-yr)	Technique of *P. jirovecii* detection in BAL specimens	Episode of *Pneumocystis* pneumonia	Treatment	Outcome[e]
P1	M	46	HIV infection	21	–	04-01-02	Wright-Giemsa	First	SMT-TMP[f]	+
P2	F	33	HIV infection	5	+	11-24-11	Wright-Giemsa; IFA[g]	First	SMT-TMP	+
P3	F	30	HIV infection	3	–	01-12-12	Wright-Giemsa	First	SMT-TMP	+
P4	F	33	HIV infection	25	–	02-16-12	Wright-Giemsa	First	SMT-TMP	+
P5	F	57	HIV infection	61	–	05-07-12	IFA	First	SMT-TMP	+
P6	F	47	HIV infection	9	NA[h]	09-29-12	Wright-Giemsa; IFA	NA	SMT-TMP	NA
P7	F	33	HIV infection	9	+	10-04-12	Wright-Giemsa; IFA	First	SMT-TMP	+
C1	M	41	HIV infection	27	–	10-03-07	Wright-Giemsa; IFA	First	SMT-TMP	+
C2	F	73	Lymphoma	ND[i]	–	02-07-08	Wright-Giemsa; IFA	First	SMT-TMP	+
C3	M	34	HIV infection	54	–	11-26-08	Wright-Giemsa; IFA	First	SMT-TMP	+
C4	F	42	Liver transplant	ND	–	06-29-09	Wright-Giemsa; IFA	First	SMT-TMP	+
C5	F	65	Bronchial carcinoma	ND	–	07-14-09	Wright-Giemsa; IFA	First	SMT-TMP	+
C6	M	26	Acute Myeloid Leukemia	ND	–	11-13-09	Wright-Giemsa; IFA	First	SMT-TMP, Atovaquone, Pentamidine	–
C7	M	60	Glioblastoma	ND	–	11-21-09	Wright-Giemsa; IFA	First	SMT-TMP	+

[a] Patients numbered from P1 to P7 were monitored in the Andrée Rosemon Hospital, Cayenne, French Guiana; patients numbered from C1 to C7 were monitored in the Brest university Hospital, Brest, France, and represent a control patient group.
[b] M, male, F, female.
[c] Past history of sulfonamide treatment over the three months preceding bronchoalveolar sampling, +, yes, –, No.
[d] Bronchoalveolar lavage.
[e] +, improvement, –, deterioration.
[f] Sulfamethoxazole-trimethoprim.
[g] IFA, indirect immunofluorescence assay (MonofluoKit *Pneumocystis jirovecii* BioRad Marnes-La-Coquette, France).
[h] Not available.
[i] Not done.