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Pneumocystis jiroveci Internal Transcribed Spacer Types in Patients Colonized by the Fungus and in Patients with Pneumocystosis from the Same French Geographic Region†

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***Pneumocystis jiroveci* (human-derived *Pneumocystis*) infections can display a broad spectrum of clinical presentations, of which pulmonary colonization with the fungus may represent an important part, occurring frequently in patients with various underlying diseases and presenting alternative diagnoses of acute pneumocystosis (*Pneumocystis carinii* pneumonia [PCP]). There are few data concerning the *P. jiroveci* genotypes involved in pulmonary colonization, whereas several genotypes responsible for PCP in immunocompromised patients have been described. In this study, *P. jiroveci* genotypes have retrospectively been investigated and compared in 6 colonized patients and in 11 patients with PCP who were in the same hospital. Seventeen archival bronchoalveolar lavage samples were genotyped at internal-transcribed spacer 1 (ITS1) and ITS2 of the nuclear rRNA operon. Fourteen different genotypes were identified, of which 1 was found only in colonized patients, 10 were found only in patients with PCP, and 3 were found in both patient populations. Mixed infections were diagnosed in 2 of the 6 colonized patients and in 6 of the 11 patients with PCP. The results show that similar genotypes can be responsible for PCP as well as pulmonary colonization. There is a high diversity of genotypes in colonized patients and in patients with PCP. Mixed infections may occur in these two patient populations. These shared features of *P. jiroveci* ITS genotypes in colonized patients and patients with PCP suggest that human populations infected by *P. jiroveci*, whatever the clinical manifestation, may play a role as a common reservoir for the fungus.**

Over the past decade, the use of PCR has led to the detection of low numbers of *Pneumocystis jiroveci* (human-derived *Pneumocystis*) (9, 36) organisms in bronchoalveolar lavage (BAL) specimens from patients, showing an alternative diagnosis of acute *Pneumocystis carinii* pneumonia (PCP) (24, 30, 40). These low levels of *P. jiroveci* organisms were considered to reflect pulmonary colonization. These cases of colonization that have been described have occurred mainly in immunocompromised patients (24, 30) and less frequently in immunocompetent patients; nevertheless, cases of colonization in immunocompromised patients presenting with underlying pulmonary diseases have been described (4, 34). Cases of pulmonary colonization with *P. jiroveci* are frequently missed since PCR assays are not usually used for the routine detection of *P. jiroveci* in pulmonary specimens. Moreover, the small numbers of *P. jiroveci* organisms carried by colonized patients render molecular typing of the organisms difficult. For these reasons, little is known concerning the types of *P. jiroveci* carried by colonized patients whereas several types of *P. jiroveci* isolates obtained from patients developing PCP have been described (5, 14–17, 19–23, 25, 27, 28, 32, 37–39). Two different molecular studies, one using single-strand conformation polymorphism analysis (10) and another using sequencing of internal-transcribed spacer 1 (ITS1) and ITS2 of the nuclear rRNA operon (29) on three and one specimens, respectively, have shown that the *P. jiroveci* types identified in colonized patients have been described in previous reports concerning patients with PCP. These results, suggesting that *P. jiroveci* types responsible for pulmonary colonization do not differ from those responsible for PCP, need further validation. Particularly, it is not known if the prevalence and diversity of the *P. jiroveci* types are comparable in colonized patients and patients with PCP who are living in the same geographic location. In this study, we have identified and compared the *P. jiroveci* ITS types present in archival BAL specimens from 6 patients colonized by the fungus and from 11 patients developing PCP who were monitored in the same hospital. *P. jiroveci* ITS types identified in both patient populations were compared with those previously described in reports concerning patients with acute PCP (16, 23, 27, 28, 37–39).

(This work was partially presented in a conference report [31]).

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† This study is dedicated to the memory of A. E. Wakefield, who contributed so much to the knowledge of *Pneumocystis* epidemiology.

MATERIALS AND METHODS

Patients and *P. jiroveci* isolates. The 17 patients from whom *P. jiroveci* isolates were obtained and typed were monitored in the University Hospital of Amiens, Amiens, France, between March 1996 and March 1999. Clinical and biological data for each patient are detailed in Table 1.

Six *P. jiroveci* isolates were obtained from six human immunodeficiency virus

TABLE 1. Characteristics of 6 patients colonized by *P. jiroveci* and 11 patients who developed PCP, for all of whom the fungus detected in BAL fluids was typed

Patient no.	Age	Sex[a]	Date of BAL (mo-day-yr)	Underlying condition(s)	T-cell count[b]		Clinical presentation of *P. jiroveci* infection (alternative diagnosis of PCP, duration of follow-up without PCP)
					$CD4^+/CD8^+$	$CD4^+$ (10^6/liter)	
A.6	64	F	03-06-96	Myeloma	0.97	185	Pulmonary colonization (bacterial pneumonia, 3 months)
A.9[c]	44	M	03-08-96	Panhypopituitarism	0.46	389	Pulmonary colonization (sarcoidosis, 12 months)
A.10	44	F	03-13-96	Renal transplantation	ND	ND	Pulmonary colonization (bacterial pneumonia, 12 months)
A.20	44	F	04-17-96	HIV infection	0.08	6	PCP
A.36	35	M	05-22-96	HIV infection	0.18	31	PCP
A.64	33	M	08-30-96	HIV infection	0.06	108	PCP
A.93	50	M	02-06-97	HIV infection	0.19	59	PCP
N.20[d]	85	F	05-30-97	Chronic obstructive pulmonary disease	1.8	968	Pulmonary colonization (exacerbation of chronic bronchitis, 6 months)
N.24	52	F	07-09-97	Systemic lupus erythematosus	0.60	569	Pulmonary colonization (pulmonary lymphoma, 32 months)
N.28	67	M	07-17-97	HIV infection	0.20	68	PCP
N.60	29	F	10-03-97	Systemic lupus erythematosus, long-term corticosteroid treatment	0.97	332	PCP
N.61	34	M	01-26-98	HIV infection	0.04	7	PCP
N.51	41	F	03-26-98	Chronic lymphoid leukemia	1.64	71	Pulmonary colonization (bacterial pneumonia, 16 months)
N.50	60	M	05-20-98	Pemphigus vulgaris, long-term corticosteroid treatment	0.92	638	PCP
N.69	33	M	11-27-98	HIV infection	0.09	17	PCP
N.70	35	M	02-18-99	Hepatic granulomatosis, long-term corticosteroid treatment	ND	ND	PCP
N.71	40	F	03-03-99	HIV infection	0.09	22	PCP

[a] F, female; M, male.
[b] The normal values were as follows: for $CD4^+/CD8^+$ T cells, >1; for $CD4^+$ T cells, >600). ND, not done.
[c] The case of patient A.9 was previously reported in reference 29.
[d] Patient N.20 was the only immunocompetent colonized patient.

(HIV)-negative patients who were colonized by the fungus. The patients were submitted to BAL to investigate pulmonary symptoms (abnormal chest X ray, cough) or fever. *P. jiroveci* was not detected in BAL specimens by microscopic examination, whereas it was detected by a PCR assay performed after rapid DNA extraction (GeneReleaser; BioVentures, Inc., Murfreesboro, Tenn.) (33) with specific primers of the gene encoding the mitochondrial large subunit rRNA (mt LSU rRNA) (40). Alternative diagnoses of PCP were available for five patients, while symptom etiology was not clearly determined for the sixth patient, who presented exacerbation of chronic bronchitis. Clinical improvement was observed in the six patients after *P. jiroveci* detection despite the absence of specific treatment for the fungus. For these reasons, the patients were considered to be merely colonized by *P. jiroveci*.

Eleven other *P. jiroveci* isolates were obtained from 11 patients diagnosed with PCP according to criteria described for HIV-infected patients by the Centers for Disease Control and Prevention (3). *P. jiroveci* was detected in BAL specimens by microscopic examination with methanol-Giemsa stain and an immunofluorescence assay (MonofluoKit *Pneumocystis*; Bio-Rad, Marnes La Coquette, France). The underlying conditions were HIV infection or long-term corticosteroid therapy. Specimens from both the colonized and PCP patients were stored at −80°C until typing.

***P. jiroveci* typing.** Typing of *P. jiroveci* isolates was performed by analysis of the ITS1 and ITS2 sequences. A DNA extraction procedure using a commercially available kit (QIAamp DNA MiniKit; Qiagen, Valencia, Calif.) was performed on all BAL specimens. To amplify the ITS1 and ITS2 sequences, a nested PCR assay was performed with two pairs of primers specific for *P. jiroveci*, N18SF (5′-GGT CTT CGG ACT GGC AGC-3′) with N26SRX (5′-TTA CTA AGG GAA TCC TTG TTA-3′) (first PCR round) and ITSF3 (5′-CTG CGG AAG GAT CAT TAG AAA-3′) with ITS2R3 (5′-GAT TTG AGA TTA AAA TTC TTG-3′) (second PCR round), described by Tsolaki et al. (37, 38). The two rounds were performed under the same reagent conditions. The primers were used (1 μM each) in a reaction mixture (50 μl) containing 3 mM $MgCl_2$, 10 mM Tris HCl, a 200 μM concentration of each deoxynucleoside triphosphate (dNTP set; Eurogentec, Seraing, Belgium), and 0.4 U of polymerase (Goldstar DNA polymerase; Eurogentec). The second PCR round was done with 3 μl of the first-round PCR products. The two rounds of PCR were done with denaturation at 94°C for 1.30 min, annealing at 55°C for 1.30 min, and extension at 72°C for 2 min for 40 cycles. The first- and second-round PCR products were electrophoresed on a 1.5% agarose gel containing ethidium bromide to visualize the expected bands (580- and 530-bp bands, respectively). To avoid contamination due to environmental amplicons, extraction, reagent preparation, and amplification procedures were performed in separate rooms with different sets of micropipettes and using barrier tips. Extraction and reagent preparations were performed in flow cabinets. Rooms required for amplified DNA manipulation were continuously submitted to an airflow with UV decontamination (SPRW 30 GR4; Paragerm, Inc., Paris, France). To monitor for possible contamination, negative controls (ultra pure distilled water) were included in extraction and the first- and second-round PCR procedures.

The second-round PCR products were purified by using Microcon PCR columns (Millipore, Bedford, Mass.) and cloned into the plasmid pGEMT (pGEMT Vector System II kit; Promega Corporation, Madison, Wis.). In order to verify if the transformation was related to the correct recombinant plasmids, each positive clone was subjected to a PCR assay using T7 (5′-GTA ATA CGA CTC ACT ATA G-3′) and SP6 (5′-ATT TAG GTG ACA CTA TAG AA-3′), universal primers specific for the T7 and SP6 promoters flanking the cloning region. Recombinant plasmids were sequenced from the two strands by using the same universal primers with the dideoxy chain termination method and an automatic sequencer (BigDye Terminator method, model 3700 sequencer; Applied Biosystems, Foster City, Calif.). Sequence alignment was performed with Clustal W software version 1.81. ITS1 and ITS2 alleles were identified by using the score previously described by Tsolaki et al. (37, 38). *P. jiroveci* ITS types were defined by a combination of the alleles of the two loci.

Nucleotide sequence accession number. The nucleotide sequence of the new ITS2 allele has been deposited in GenBank under accession number AF498265.

RESULTS

Results of the *P. jiroveci* ITS type identification are shown in Table 2. By applying the score described for typing by Tsolaki et al. (37, 38), four different types were identified in six colo-

TABLE 2. Identification of *P. jiroveci* ITS types in 6 patients colonized by the fungus and in 11 patients who developed PCP in the University Hospital, Amiens, France

Patient no.	Clone no.	Nucleotide(s) at indicated position(s) in locus[a]:										Type(s)	C[b] or PCP
		ITS1				ITS2							
		2	16	74–75	111–113	54–56	63	67–71	122	169–172	176		
A.6	A6.4	T	A	AG	TTA	TAA	-	AATAT	-	----	G	B_2a_1	C
A.9	A9.1	T	T	--	TTA	TAA	–	AATAA	–	----	A	'A'a_3[c]	C
	A9.2	T	T	--	TTA	TAA	–	AATAA	–	----	A	'A'a_3	
	A9.3	T	T	--	TTA	TAA	–	AATAA	–	----	A	'A'a_3	
A.10	A10.1	T	T	AG	TTA	TAA	–	AATAA	–	----	A	B_1a_3	C
	A10.3	T	T	AG	TTA	TAA	–	AATAA	–	----	A	B_1a_3	
	A10.5	T	T	AG	TTA	TAA	–	AATAA	–	----	A	B_1a_3	
A.20	A20.1	T	T	--	TTA	TAA	–	AATAA	–	----	A	'A'$_3$	PCP
	A20.2	T	T	AG	TTA	TAA	–	AATAA	–	----	A	B_1a_3	
A.36	A36.2	T	A	AG	TTA	TAA	–	AATAT	–	----	G	B_2a_1	PCP
	A36.3	T	A	AG	TTA	TAA	–	AATAT	–	----	G	B_2a_1	
	A36.7	T	A	AG	TTA	TAA	–	AATAT	–	----	G	B_2a_1	
A.64	A64.1	C	T	--	TTA	TAA	–	AATAT	–	----	A	A_2a_4	PCP
	A64.2	T	A	--	TTA	TAA	–	AATAT	–	----	A	A_3a_4	
	A64.3	C	T	--	TTA	---	A	-----	–	----	A	A_2c_1	
A.93	A93.2	T	A	AG	TTA	TAA	–	AATAT	–	----	G	B_2a_1	PCP
	A93.3	T	A	AG	TTA	TAA	–	AATAT	–	----	G	B_2a_1	
	A93.6	T	A	AG	TTA	TAA	–	AATAA	–	----	A	B_2a_3	
	A93.8	T	A	AG	TTA	TAA	–	AATAT	–	----	G	B_2a_1	
N.20	N20.2	T	A	AG	TTA	TAA	–	AATAT	–	----	G	B_2a_1	C
	N20.7	T	A	AG	TTA	TAA	–	AATAT	–	----	G	B_2a_1	
N.24	N24.1	T	T	AG	TTA	TAA	–	AATAA	–	----	A	B_1a_3	C
	N24.2	T	T	AG	TTA	TAA	–	AATAA	–	----	A	B_1a_3	
	N24.4	T	A	--	TTA	TAA	–	AATAA	–	----	A	A_3a_3	
N.28	N28.1	T	T	AG	TTA	TAA	–	AATAT	C	--AT	G	B_1b_2	PCP
	N28.9	T	T	AG	TTA	TAA	–	AATAT	C	--AT	G	B_1b_2	
	N28.10	T	T	AG	TTA	TAA	–	AATAT	C	--AT	G	B_1b_2	
N.60	N60.3	T	T	AG	TTA	TAA	–	AATAA	–	----	A	B_1a_3	PCP
	N60.6	T	T	AG	TTA	TAA	–	AATAA	–	----	A	B_1a_3	
	N60.9	T	T	AG	TTA	TAA	–	AATAA	–	----	A	B_1a_3	
N.61	N61.6	C	T	--	TTA	---	A	-----	–	----	A	A_2c_1	PCP
	N61.7	C	T	--	TTA	---	A	-----	–	----	A	A_2c_1	
	N61.10	C	T	--	TTA	---	A	-----	–	----	A	A_2c_1	
N.51	N51.1	T	T	AG	TTA	TAA	–	AATAA	–	----	A	B_1a_3	C
	N51.6	T	T	AG	TTA	TAA	–	AATAA	–	----	A	B_1a_3	
	N51.7	T	A	--	TTA	TAA	–	AATAA	–	----	A	A_3a_3	
N.50	N50.2	T	A	AG	TTA	TAA	–	AATAT	–	----	G	B_2a_1	PCP
	N50.5	T	A	AG	TTA	TAA	–	AATAT	–	----	G	B_2a_1	
N.69	N69.1	C	T	--	TTA	---	A	-----	–	----	A	A_2c_1	PCP
	N69.2	C	T	--	TTA	TAA	–	AATAT	–	----	A	A_2a_4	
	N69.4	C	T	--	TTA	TAA	–	AATAT	–	--AT	A	A_2'b'[d]	
N.70	N70.2	T	A	AG	TTA	TAA	–	AATAA	–	----	A	B_2a_3	PCP
	N70.3	T	T	AG	TTA	TAA	–	AATAT	–	--AT	G	B_1b_1	
	N70.4	T	A	AG	TTA	TAA	–	AATAT	–	--AT	G	B_2b_1	
N.71	N71.1	T	A	--	TTA	TAA	–	AATAA	–	----	G	A_3a_2	PCP
	N71.2	T	T	--	TTA	TAA	–	AATAA	–	----	A	'A'a_3	
	N71.3	T	T	--	TTA	TAA	–	AATAA	–	----	A	'A'a_3	
	N71.4	T	T	--	TTA	TAA	–	AATAA	–	----	G	'A'a_2	
	N71.5	T	A	AG	TTA	TAA	–	AATAT	–	----	G	B_2a_1	

[a] Scoring positions are described in references 37 and 38; complete sequence data are available upon request from the authors. Each dash represents one position for which the nucleotide is not specified.
[b] C, colonized.
[c] The ITS1 allele designated 'A' differs from the A_2 and A_3 previously described (37, 38) by having T residues at positions 2 and 16.
[d] The ITS2 allele designated 'b' (GenBank accession number AF498265) differs from the b_1 previously described (38) by having an A residue at position 176.

nized patients. Type B_1a_3 was detected in three patients (50%). Types B_2a_1 and A_3a_3 were each found in two patients (33%). Type A_3a_3 results in a new combination of the ITS1 allele A_3 with the ITS2 allele a_3 described previously (37, 38). This new allele combination was considered to reflect a new type. The ITS1 allele, which we designated 'A,' was close to allele A_2 or A_3; however, it differed by T residues at positions 2 and 16 of the ITS1 locus. Applying the score described for typing by Lee et al. (23), type 'A'a_3 is the same as type Gg (GenBank accession numbers AF013812 [ITS1G] and AF013827 [ITS2g]). More than one type was detected in two patients, showing the same type association, A_3a_3/B_1a_3.

Thirteen different types were found in 11 patients with PCP. Type B_2a_1 was found in four patients (36%). Type A_2c_1 was identified in three patients (27%). Types B_1a_3, B_2a_3, A_2a_4, and 'A'a_3 were each detected in two different patients (18%). Types A_3a_4, B_1b_2, and A_2'b' were each identified in only one patient. Types B_1b_1 and B_2b_1 were found in the same patient. Both types A_3a_2 and 'A'a_2 were found in another patient. Types B_1a_3, B_2a_1, A_2c_1, B_1b_2, B_1b_1, and B_2a_3 have been previously reported in patients with PCP (16, 27, 28, 37–39). Type B_2b_1, which was not previously observed by Tsolaki et al. (37, 38), has previously been described by Lee et al. and designated Nb (23). Types A_2a_4, A_3a_4, and A_3a_2 result from new combinations of ITS1 and ITS2 alleles described previously (37, 38). These new allele combinations were considered to be new types. Type 'A'a_2 results in a combination of the identical ITS1 allele 'A' described above with the ITS2 allele a_2 described previously (37). The ITS2 allele that we designated 'b' differed from the b_1 allele previously reported (37) by an A residue at position 176 of the ITS2 locus. Allele combinations 'A'a_2 and A_2'b' were also considered to be new types. More than one type was found in six patients with PCP, showing six different type associations.

DISCUSSION

In this study, specimens from 6 colonized patients and 11 patients who had PCP were typed. The hypothesis that low levels of *P. jiroveci* initially detected only by PCR in the six colonized patients can be related to sample contamination by the fibroscopes previously used for other patients who had PCP was ruled out. Indeed, a PCP case was diagnosed 16 weeks before the first colonized patient enrolled in the study (data not shown). The other PCP cases were diagnosed at least 8 weeks before each case of pulmonary colonization with *P. jiroveci* (Table 1). Positive results of *P. jiroveci* detection in the six colonized patients were obtained by using two PCR assays performed with specific primers from two different loci (at the mt LSU rRNA and the ITS regions) after two different extraction procedures (with the GeneReleaser and QIAamp DNA MiniKit). As different rooms, flow cabinets, and air UV decontamination procedures as well as several negative controls were used, contamination of specimens within PCR processing was excluded. These data are consistent with a genuine presence of *P. jiroveci* cells in the respiratory tracts of the patients.

Five colonized patients had an impairment of immunity. Thus, the presence of *P. jiroveci* was consistent with their immune status (24, 30), the fungus behaving opportunistically. In the sixth patient, who was immunocompetent and had chronic bronchitis, the presence of *P. jiroveci* may have been due to lung damage, as previously suggested by Armbruster et al. (4).

For the typing, the method based on ITS sequence analysis was chosen since these loci are more informative than the mt LSU rRNA (37). By using this method, previous reports have shown that the most frequent *P. jiroveci* types in patients with PCP from different regions of Europe or the United States (23) were identical (B_2a_1 and B_1a_3 [corresponding to Ne and Eg as described by Lee et al. {23}]). This identity of major types among nonepidemiologically linked isolates pleads in favor of ITS sequence analysis as a valuable method for typing the *P. jiroveci* cells involved in PCP. Our results show that this method also makes it possible to type organisms involved in pulmonary colonization. Nevertheless, ITS genotyping is quite difficult for colonized-patient samples that contain small quantities of *P. jiroveci*. Even after amplification by ITS PCR, the amount of *P. jiroveci* DNA obtained from colonized-patient samples remained small. For this reason, a prior cloning of ITS PCR products was preferred to direct sequencing in order to enhance the amount of DNA to be sequenced. Furthermore, cloning facilitates the detection of mixed infections revealed by the identification of more than one type in specimens.

It has recently been established that genotyping by PCR on BAL specimens may represent only the dominant types present within lungs (15). Consistently, our observations may also concern only dominant types in both PCP and colonized patients. In the present work, a high diversity of *P. jiroveci* ITS genotypes was observed in colonized patients close to that previously reported in studies on patients with PCP (28, 38). Nonetheless, the diversity of types in colonized patients appears to be lower than that in our patients with PCP. The results are linked to the low rate of mixed infections in the first patient population in comparison with that in the second one. This difference may be partially due to the fact that the sequencing of only one clone in one colonized patient (A.6) did not allow the detection of a mixed infection. Nonetheless, our results establish that mixed infections may occur in patients with pulmonary colonization and that this form of *P. jiroveci* infection may not be necessarily clonal as previously suggested for PCP (2, 38).

Among the four types identified in the colonized patients, three types (B_1a_3, B_2a_1, and 'A'a_3) were also detected in our patients with PCP. It is noteworthy that types B_1a_3 and B_2a_1, two of the most frequent types previously identified in reports on patients who had PCP (28, 37–39), are also common in the two patient populations examined in our study. Type A_3a_3 was found only in colonized patients. As this type has not previously been reported, the hypothesis that it can be involved only in pulmonary colonization cannot be ruled out. Likewise, the fact that type A_2c_1, found in patients with PCP (references 28 and 37 and this study), was absent in colonized patients may be consistent with the results of Miller and Wakefield (28), who suggested that this type was responsible for severe PCP. In addition to type A_2c_1, nine other less frequent types (A_2a_4, B_2a_3, A_3a_4, B_1b_2, A_2'b', B_1b_1, B_2b_1, A_3a_2, and 'A'a_2) were detected only in patients with PCP, showing a high diversity of types in this patient population. In fact, this diversity of *P. jiroveci* types, as well as the occurrence of mixed infections in the two patient populations, renders it quite difficult to clearly establish a correlation between genotypes and clinical profiles

(colonization versus PCP). The difficulties have previously been addressed to explain the conflicting results of previous studies on clinical correlation with *P. jiroveci* types in patients with PCP (11–14, 28). Our results indicate above all that shared features of *P. jiroveci* ITS types (diversity, partial commonality of types, and occurrence of mixed infections) can be observed in both colonized individuals and patients with PCP, monitored in the same geographic region at the same time.

Host-to-host airborne transmission of the fungus has been demonstrated in rodent models (7, 18, 35), and observations suggest that interindividual transmission occurs in man (26, 27). Moreover, it is now presently assumed that *Pneumocystis* organisms infecting mammalian hosts are host specific (1, 8). Consistently, an animal reservoir for *P. jiroveci* has been excluded. Although an exosaprophytic form of *P. jiroveci* cannot be ruled out, these data suggest that humans constitute the main reservoir and infection sources of *P. jiroveci.* New detection tools like PCR assays have revealed that *P. jiroveci* infection can display a large spectrum of clinical presentations, of which acute PCP in immunocompromised patients may represent only a small part, while pulmonary colonization may be a major part (6). The results of our *P. jiroveci* ITS type identification in colonized patients with those of previous studies on patients with PCP suggest that human populations infected by *P. jiroveci*, whatever the clinical context, may play a role as a common human reservoir.

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REFERENCES

1. **Aliouat, E. M., E. Mazars, E. Dei-Cas, P. Delcourt, P. Billaut, and D. Camus.** 1994. *Pneumocystis* cross infection experiments using SCID mice and nude rats as recipient host, showed strong host-species specificity. J. Eukaryot. Microbiol. **41**(Suppl.)**:**71S.
2. **Ambrose, H. E., C. A. Ponce, A. E. Wakefield, R. F. Miller, and S. L. Vargas.** 2001. Distribution of *Pneumocystis carinii* f. sp. *hominis* types in the lung of a child dying of *Pneumocystis* pneumonia. Clin. Infect. Dis. **33:**E100–E102. [Online.]
3. **Anonymous.** 1992. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. Recommended report. Morb. Mortal. Wkly. Rep. **41:**1–19.
4. **Armbruster, C., A. Hassl, and S. Kriwanek.** 1997. *Pneumocystis carinii* colonization in the absence of immunosuppression. Scand. J. Infect. Dis. **29:** 591–593.
5. **Atzori, C., E. Angeli, F. Agostoni, A. Mainini, V. Micheli, and A. Cargnel.** 1998. Biomolecular techniques to detect *Pneumocystis carinii* f. sp. *hominis* pneumonia in patients with acquired immunodeficiency syndrome. Int. J. Infect. Dis. **3:**76–81.
6. **Dei-Cas, E.** 2000. *Pneumocystis* infections: the iceberg? Med. Mycol. **38** (Suppl. 1)**:**23–32.
7. **Dumoulin, A., E. Mazars, N. Seguy, D. Gargallo-Viola, S. Vargas, J. C. Cailliez, E. M. Aliouat, A. E. Wakefield, and E. Dei-Cas.** 2000. Transmission of *Pneumocystis carinii* disease from immunocompetent contacts of infected hosts to susceptible hosts. Eur. J. Clin. Microbiol. Infect. Dis. **19:**671–678.
8. **Durand-Joly, I., E.-M. Aliouat, C. Recourt, K. Guyot, N. François, M. Wauquier, D. Camus, and E. Dei-Cas.** 2002. *Pneumocystis carinii* f. sp. *hominis* is not infectious for SCID mice. J. Clin. Microbiol. **40:**1862–1865.
9. **Frenkel, J. K.** 1976. *Pneumocystis jiroveci* n. sp. from man: morphology, physiology, and immunology in relation to pathology. Natl. Cancer Inst. Monogr. **43:**13–30.
10. **Hauser, P. M., D. S. Blanc, J. Bille, A. Nahimana, and P. Francioli.** 2000. Carriage of *Pneumocystis carinii* by immunosuppressed patients and molecular typing of the organisms. AIDS **14:**461–463.
11. **Hauser, P. M., D. S. Blanc, P. Sudre, E. Senggen Manoloff, A. Nahimana, J. Bille, R. Weber, and P. Francioli.** 2001. Genetic diversity of *Pneumocystis carinii* in HIV-positive and -negative patients as revealed by PCR-SSCP typing. AIDS **15:**461–466.
12. **Hauser, P. M., D. S. Blanc, A. Telenti, A. Nahimana, J. Bille, and P. Francioli.** 1998. Potential coinfections complicate typing of *Pneumocystis carinii* f. sp. *hominis*. J. Clin. Microbiol. **36:**3111.
13. **Hauser, P. M., P. Sudre, A. Nahimana, and P. Francioli.** 2001. Prophylaxis failure is associated with a specific *Pneumocystis carinii* genotype. Clin. Infect. Dis. **33:**1080–1082.
14. **Helweg-Larsen, J., C. H. Lee, S. Jin, J. Y. Hsueh, T. L. Benfield, J. Hansen, J. D. Lundgren, and B. Lundgren.** 2001. Clinical correlation of variations in the internal transcribed spacer regions of rRNA genes in *Pneumocystis carinii* f. sp. *hominis*. AIDS **15:**451–459.
15. **Helweg-Larsen, J., B. Lundgren, and J. D. Lundgren.** 2001. Heterogeneity and compartmentalization of *Pneumocystis carinii* f. sp. *hominis* genotypes in autopsy lungs. J. Clin. Microbiol. **39:**3789–3792.
16. **Helweg-Larsen, J., A. G. Tsolaki, R. F. Miller, B. Lundgren, and A. E. Wakefield.** 1998. Clusters of *Pneumocystis carinii* pneumonia: analysis of person-to-person transmission by genotyping. Q. J. Med. **91:**813–820.
17. **Hosoya, N., T. Takahashi, M. Wada, T. Endo, T. Nakamura, H. Sakashita, K. Kimura, K. Ohnishi, Y. Nakamura, T. Mizuochi, and A. Iwamoto.** 2000. Genotyping of *Pneumocystis carinii* f. sp. *hominis* isolates in Japan based on nucleotide sequence variations in internal transcribed spacer regions of rRNA genes. Microbiol. Immunol. **44:**591–596.
18. **Hughes, W. T., D. L. Bartley, and B. M. Smith.** 1983. A natural source of infection due to *Pneumocystis carinii*. J. Infect. Dis. **147:**595.
19. **Keely, S. P., and J. R. Stringer.** 1997. Sequences of *Pneumocystis carinii* f. sp. *hominis* strains associated with recurrent pneumonia vary at multiple loci. J. Clin. Microbiol. **35:**2745–2747.
20. **Latouche, S., E. Ortona, E. Mazars, P. Margutti, E. Tamburrini, A. Siracusano, K. Guyot, M. Nigou, and P. Roux.** 1997. Biodiversity of *Pneumocystis carinii hominis*: typing with different DNA regions. J. Clin. Microbiol. **35:** 383–387.
21. **Latouche, S., J. L. Poirot, C. Bernard, and P. Roux.** 1997. Study of internal transcribed spacer and mitochondrial large-subunit genes of *Pneumocystis carinii hominis* isolated by repeated bronchoalveolar lavage from human immunodeficiency virus-infected patients during one or several episodes of pneumonia. J. Clin. Microbiol. **35:**1687–1690.
22. **Latouche, S., J. L. Poirot, E. Maury, V. Bertrand, and P. Roux.** 1997. *Pneumocystis carinii hominis* sequencing to study hypothetical person-to-person transmission. AIDS **11:**549.
23. **Lee, C. H., J. Helweg-Larsen, X. Tang, S. Jin, B. Li, M. S. Bartlett, J. J. Lu, B. Lundgren, J. D. Lundgren, M. Olsson, S. B. Lucas, P. Roux, A. Cargnel, C. Atzori, O. Matos, and J. W. Smith.** 1998. Update on *Pneumocystis carinii* f. sp. *hominis* typing based on nucleotide sequence variations in internal transcribed spacer regions of rRNA genes. J. Clin. Microbiol. **36:**734–741.
24. **Leigh, T. R., H. O. Kangro, B. G. Gazzard, D. J. Jeffries, and J. V. Collins.** 1993. DNA amplification by the polymerase chain reaction to detect subclinical *Pneumocystis carinii* colonization in HIV-positive and HIV-negative male homosexuals with and without respiratory symptoms. Respir. Med. **87:**525–529.
25. **Lu, J. J., M. S. Bartlett, J. W. Smith, and C. H. Lee.** 1995. Typing of *Pneumocystis carinii* strains with type-specific oligonucleotide probes derived from nucleotide sequences of internal transcribed spacers of rRNA genes. J. Clin. Microbiol. **33:**2973–2977.
26. **Miller, R. F., H. E. Ambrose, V. Novelli, and A. E. Wakefield.** 2002. Probable mother-to-infant transmission of *Pneumocystis carinii* f. sp. *hominis* infection. J. Clin. Microbiol. **40:**1555–1557.
27. **Miller, R. F., H. E. Ambrose, and A. E. Wakefield.** 2001. *Pneumocystis carinii* f. sp. *hominis* DNA in immunocompetent health care workers in contact with patients with *P. carinii* pneumonia. J. Clin. Microbiol. **39:**3877–3882.
28. **Miller, R. F., and A. E. Wakefield.** 1999. *Pneumocystis carinii* genotypes and severity of pneumonia. Lancet **353:**2039–2040.
29. **Nevez, G., K. Guyot, A. Totet, C. Raccurt, and E. Dei-Cas.** 2001. Pulmonary colonisation with *Pneumocystis carinii* in an immunosuppressed HIV-negative patient: detection and typing of the fungus by PCR. J. Med. Microbiol. **50:**198–200.
30. **Nevez, G., C. Raccurt, P. Vincent, V. Jounieaux, and E. Dei-Cas.** 1999. Pulmonary colonization with *Pneumocystis carinii* in human immunodeficiency virus-negative patients: assessing risk with blood CD4+ T cell counts. Clin. Infect. Dis. **29:**1331–1332.
31. **Nevez, G., A. Totet, and C. Raccurt.** 2001. Typing of *Pneumocystis carinii* f. sp. *hominis* in patients with or without pneumocystosis. J. Eukaryot. Microbiol. **48**(Suppl.)**:**124S-125S.
32. **Ortiz-Rivera, M., and M. J. Leibowitz.** 1997. Partial sequence of the rRNA operon of *Pneumocystis carinii* isolates from Puerto Rico. P. R. Health Sci. J. **16:**251–254.
33. **Rabodonirina, M., D. Raffenot, L. Cotte, A. Boibieux, M. Mayencon, G. Bayle, F. Persat, F. Rabatel, C. Trepo, D. Peyramond, and M. A. Piens.** 1997. Rapid detection of *Pneumocystis carinii* in bronchoalveolar lavage specimens from human immunodeficiency virus-infected patients: use of a simple DNA extraction procedure and nested PCR. J. Clin. Microbiol. **35:**2748–2751.

34. **Sing, A., A. Roggenkamp, I. B. Autenrieth, and J. Heesemann.** 1999. *Pneumocystis carinii* carriage in immunocompetent patients with primary pulmonary disorders as detected by single or nested PCR. J. Clin. Microbiol. **37:** 3409–3410.
35. **Soulez, B., F. Palluault, J. Y. Cesbron, E. Dei-Cas, A. Capron, and D. Camus.** 1991. Introduction of *Pneumocystis carinii* in a colony of SCID mice. J. Protozool. **38**(Suppl.)**:**123–125.
36. **Stringer, J. R., C. B. Beard, R. F. Miller, and A. E. Wakefield.** 2002. A new name (*Pneumocystis jiroveci*) for *Pneumocystis* from humans. Emerg. Infect. Dis. **8:**891–896.
37. **Tsolaki, A. G., P. Beckers, and A. E. Wakefield.** 1998. Pre-AIDS era isolates of *Pneumocystis carinii* f. sp. *hominis*: high genotype similarity with contemporary isolates. J. Clin. Microbiol. **36:**90–93.
38. **Tsolaki, A. G., R. F. Miller, A. P. Underwood, S. Banerji, and A. E. Wakefield.** 1996. Genetic diversity at the internal transcribed spacer regions of the rRNA operon among isolates of *Pneumocystis carinii* from AIDS patients with recurrent pneumonia. J. Infect. Dis. **174:**141–156.
39. **Tsolaki, A. G., R. F. Miller, and A. E. Wakefield.** 1999. Oropharyngeal samples for genotyping and monitoring response to treatment in AIDS patients with *Pneumocystis carinii* pneumonia. J. Med. Microbiol. **48:**897–905.
40. **Wakefield, A. E., F. J. Pixley, S. Banerji, K. Sinclair, R. F. Miller, E. R. Moxon, and J. M. Hopkin.** 1990. Detection of *Pneumocystis carinii* with DNA amplification. Lancet **336:**451–453.