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Update on *Pneumocystis carinii* f. sp. *hominis* Typing Based on Nucleotide Sequence Variations in Internal Transcribed Spacer Regions of rRNA Genes

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***Pneumocystis carinii* f. sp. *hominis* isolates from 207 clinical specimens from nine countries were typed based on nucleotide sequence variations in the internal transcribed spacer regions I and II (ITS1 and ITS2, respectively) of rRNA genes. The number of ITS1 nucleotides has been revised from the previously reported 157 bp to 161 bp. Likewise, the number of ITS2 nucleotides has been changed from 177 to 192 bp. The number of ITS1 sequence types has increased from 2 to 15, and that of ITS2 has increased from 3 to 14. The 15 ITS1 sequence types are designated types A through O, and the 14 ITS2 types are named types a through n. A total of 59 types of *P. carinii* f. sp. *hominis* were found in this study.**

We have previously reported that the nucleotide sequences of the internal transcribed spacer (ITS) regions of rRNA genes of different isolates of *Pneumocystis carinii* f. sp. *hominis* are variable and that this sequence variation can be used for typing (15). There are two ITS regions in the genome of *P. carinii*: ITS region I (ITS1) is located between the 18S and 5.8S rRNA genes, and ITS2 is located between the 5.8S and 26S rRNA genes. ITS1 was found to contain 157 bp, and ITS2 was determined to be 177 bp (15). Sequence variations in the ITS1 region that were found to be useful for typing are located at positions 6, 14, 67, 76, and 77. Based on nucleotide sequence variations at these positions, ITS1 sequences were classified into two types (A and B). Type A has a C at position 6, a T at position 14, and bases missing at positions 67, 76, and 77. Type B has a T at position 6, a base missing at position 14, a T at position 67, and AG at positions 76 and 77.

In the ITS2 region, nucleotide variations at positions 50 to 52, 59, 63 to 67, 160 and 161, and 165 were used for typing, and three types of ITS2, designated types a, b, and c, were found. Types a and b differ by two bases at positions 160 and 161, where type b has residues AT and type a lacks these two bases. Type c lacks bases at positions 50 to 52, 63 to 67, and 160 and 161. In addition, it has an A instead of a G residue at position 165.

P. carinii isolates with type A or B ITS1 may have type a, b, or c ITS2; therefore, there were six potential combination types (Aa, Ab, Ac, Ba, Bb, and Bc). We have previously reported finding four types (Ac, Ba, Bb, and Bc) (15). In this study, we have expanded the sample size and found many more new types. This paper reports a very substantial revision of ITS types of *P. carinii* f. sp. *hominis.*

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MATERIALS AND METHODS

Specimens. Specimens used for this study included bronchoalveolar lavage (BAL) fluids, BAL smears on slides, and paraffin-embedded lung biopsy specimens. A total of 207 specimens from 9 countries were used including 123 from Denmark, 44 from the United States, 10 from Ivory Coast (17, 18), 9 from Italy, 6 from France, 5 from Sweden, 4 each from The Netherlands and Portugal, and 2 from Thailand (Table 1). Specimens from Denmark and Sweden were BAL smears on slides, those from Thailand and West Africa were paraffin-embedded lung biopsy specimens, and the remaining specimens were BAL fluid specimens. The specimens from the United States included 38 from Indiana University Medical Center, 4 from the University of Medicine and Dentistry of New Jersey as described previously (15), and 2 from the University of Michigan. Fresh BAL fluid specimens and smears were processed for PCR by the procedures described previously (12, 13). Paraffin-embedded tissue sections were processed for PCR as described by Greer et al. (4).

PCR. Nested PCR was performed on all specimens to obtain a sufficient amount of DNA fragment for analysis by the procedure of Lu et al. (15) with the following modifications. The first PCR was performed with primers 1724F2 (5′-AGTTGATCAAATTTGGTCATTTAGAG-3′) and ITS2R (5′-CTCGGAC GAGGATCCTCGCC-3′) under the following conditions: (i) 3 min at 94°C; (ii) 5 cycles, with 1 cycle consisting of 1.5 min at 94°C, 1.5 min at 62°C, and 1.5 min at 72°C; and (iii) 30 cycles, with 1 cycle consisting of 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C. The PCR mixture was in a total volume of 40 μl containing 50 ng of template DNA, PCR buffer, 2.5 U of *Taq* polymerase, 3 mM $MgCl_2$, 0.2 mM deoxynucleoside triphosphate, and 20 pmol of each primer. The second PCR was performed with primers FX (5′-TTCCGTAGGTGAACCTGCG-3′) and RT2 (5′-CTGATTTGAGATTAAAATTCTTG-3′) under the following conditions: (i) 3 min at 94°C; (ii) 5 cycles, with 1 cycle consisting of 1.5 min at 94°C, 1.5 min at 58°C, and 1.5 min at 72°C; and (iii) 30 cycles, with 1 cycle consisting of 1 min at 94°C, 1 min at 56°C, and 1 min at 72°C. The reaction mixture contained 2 μl of the first PCR product and the same components as those of the first PCR except that 1.5 mM $MgCl_2$ was used.

TABLE 1. Geographical sources of specimens used in this study

Source	No.
Denmark	123
United States	44
Ivory Coast	10
Italy	9
France	6
The Netherlands	4
Portugal	4
Sweden	5
Thailand	2
Total	207

Typing. Nested PCR products of ITS regions were first probed with type-specific probes 1A, 1B, 2a, 2b, and 2c to determine whether the specimen contained a single or multiple types of *P. carinii* as described previously (16). The specimens which contained a single type of *P. carinii* were sequenced directly with the Sequenase kit (Amersham Life Science, Arlington Height, Ill.), and those which contained multiple types of *P. carinii* were cloned and then sequenced. Screening of recombinant clones that contained the correct insert was achieved by colony hybridization as described previously (7) using all five of the type-specific probes mentioned above. Five colonies which reacted with a given probe were sequenced. A sequence which was found to be identical in at least three of the five colonies was considered to be a correct sequence. A sequence which appeared in at least two different specimens was considered a distinct type.

Nucleotide sequence accession numbers. All the type-specific sequences have been deposited in GenBank. The sequences and accession numbers are as follows: ITS1A, AF013806; ITS1B, AF013807; ITS1C, AF013808; ITS1D, AF013809; ITS1E, AF013810; ITS1F, AF013811; ITS1G, AF013812; ITS1H, AF013813; ITS1I, AF013814; ITS1J, AF013815; ITS1K, AF013816; ITS1L, AF013817; ITS1M, AF013818; ITS1N, AF013819; ITS1O, AF013820; ITS2a, AF013821; ITS2b, AF013822; ITS2c, AF013823; ITS2d, AF013824; ITS2e, AF013825; ITS2f, AF013826; ITS2g, AF013827; ITS2h, AF013828; ITS2i, AF013829; ITS2j, AF013830; ITS2k, AF013831; ITS2l, AF013832; ITS2m, AF013833; and ITS2n, AF013834.

RESULTS

A total of 15 ITS1 and 14 ITS2 sequence types were found from the 207 specimens that we have examined. The number of ITS1 nucleotides has been changed from 157 to 161 bp. This is due to insertions of TT between positions 22 and 23 and AT between positions 50 and 51 of the original sequence. The ITS2 sequence has been changed from 177 to 192 bp. Insertions of new bases include AA between positions 47 and 48, TAA between positions 52 and 53, T between positions 70 and 71, AT between positions 159 and 160, and AAAATAC between positions 169 and 170 of the original sequence. The nucleotides of the revised sequences have been renumbered, and new position numbers are used hereafter. The consensus nucleotide sequences of both ITS1 and ITS2 are shown in Fig. 1.

The 15 ITS1 types are designated types A through O. Types that are close to each other are named with sequential letters. For example, types A and B differ by only one base at position 12, where type A has a T and type B lacks this base. Type A is the same type A as we have reported previously (15). The original type B has been renamed type E. All the other types are novel. Not every *P. carinii* isolate has the same number of nucleotides in both ITS1 and ITS2. Some isolates are missing bases at certain positions. In ITS1, sequence variations are found at nucleotide positions 6, 12, 15, 21, 23, 24, 28, 34, 42, 53, 54, 80, 81, and 115 to 118 (Fig. 2).

Nucleotide sequence variations in ITS2 are much more extensive. They are found at positions 48 and 49, 52 and 57, 62 to 70, 72, 76, 122, 160, 166 to 171, 173, and 178 to 184 (Fig. 3). There are 14 types of ITS2 sequences, designated types a through n. Similar to ITS1 types, those that are close to each other are named with sequential letters. The original type b remains type b, whereas the original types a and c have been renamed types e and m, respectively.

There are six sequences from specimens IT007, FR004, DK029, DK594, DK323, and DK313 (Fig. 3) that have variations at variable positions of ITS2 but are not considered distinct types, because only one example of each has been found so far. The sequence found in specimen IT007 differs from that of type g at position 173, where IT007 has a G and type g has an A. The sequence of specimen FR004 differs from that of type b by only one base at position 72 where type b has a T and FR004 has an A. The sequence found in specimen DK029 is unique. It has no base deletions at positions 48 and 49. It also lacks bases at positions 55 to 57, 64, 76, 122, 168 and 169, and 178 to 184 and has an A residue at position 72, a T at position 166, and an A at position 173. The sequence found in specimen DK594 has an A at position 64 and a T at position 76; otherwise, it is identical to type b. The sequence of DK323 differs from that of type d by only one base at position 122, where it is missing the base C. The sequence of DK313 differs from type f by one base at position 72, where DK313 has an A instead of a T.

As previously described, *P. carinii* sequence types are designated with a two-letter code (15). The first letter is the ITS1 type, and the second one is the ITS2 type. Among the 207 specimens that have been typed, there are a total of 59 combination types of *P. carinii* f. sp. *hominis* (Table 2). Type Eg is

ITS1

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        10          20          30          40          50          60          70
GAAAATTCAG CTTTAAACAC TTTTCCCTAG TGTTTTAGCA TTTTTCAAAC ATATCTGTGA ATTTTTTTTT
        80          90         100         110         120         130         140
TGTTTGGCGA GGAGCTGGCT TTTTTGCTTG CCTCGCCAAA GGTGTTTATT TTTAAAATTT TAAATTGAAT
       150         160
TTCAGTTTTA GAATTTTTTA A
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ITS2

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        10          20          30          40          50          60          70
TTAAGTTCCT TTTTTCAAGC AGAAAAAAGG GGATTGGGCT TTGCAAAAAT ATAATAATTA GAAATAAAAT
        80          90         100         110         120         130         140
ATTTATTATG CATGCTAGTC TGAAATTCAA AAGTAGCTTT TTTTCTTTGC CCTAGTGTCG TAAAAATTCG
       150         160         170         180         190
CTGGGAAAGA AGGAAAAAAG CTTTTATATA TAGATACAAA ATACAAGAAT TT
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FIG. 1. Revised nucleotide sequences of ITS1 and ITS2. The number of nucleotides has been changed from the previously reported 157 bp to 161 bp for ITS1 and from 177 to 192 bp for ITS2. The sequences shown are consensus sequences; they are not the sequences of a certain type of *P. carinii* f. sp. *hominis*.

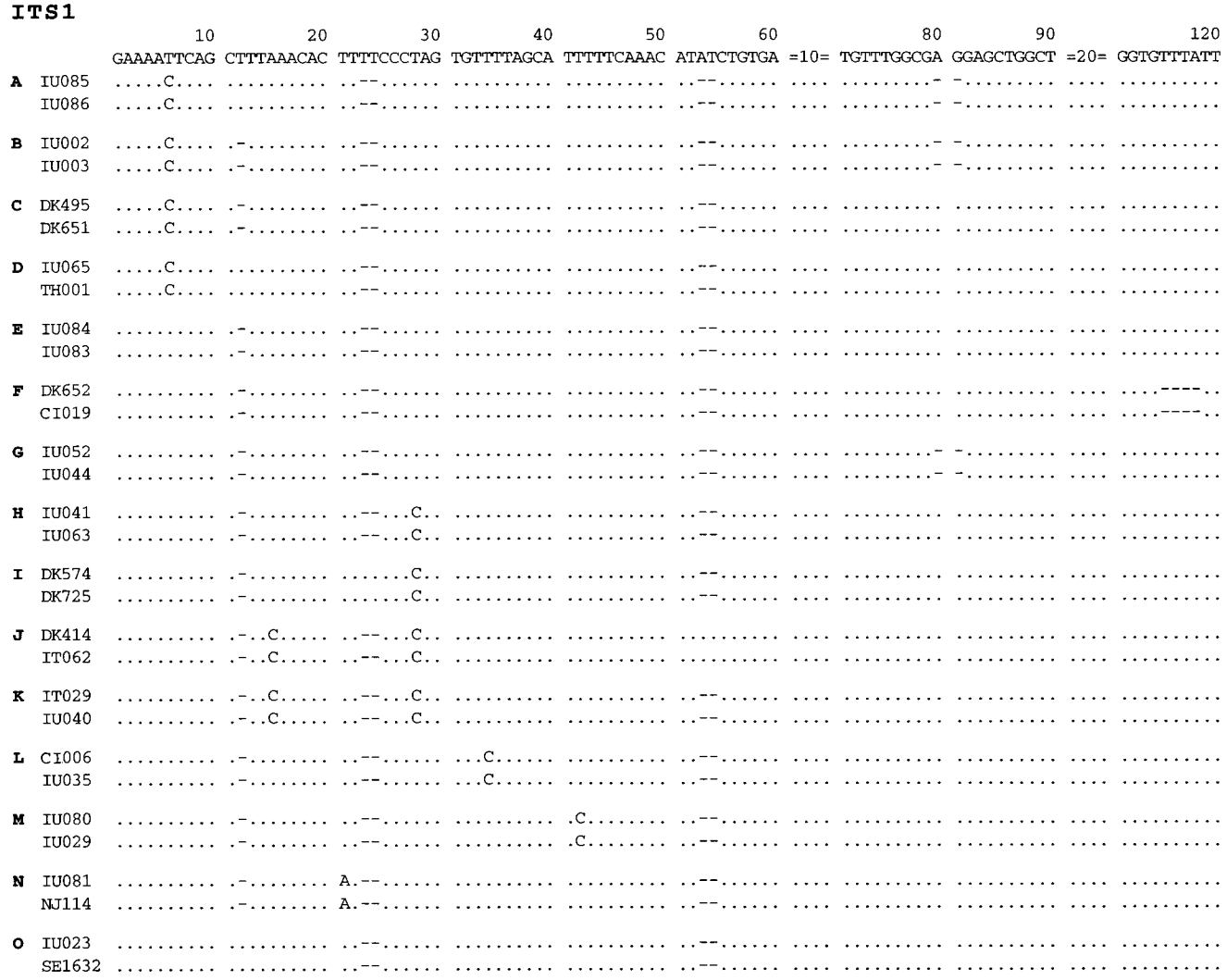

FIG. 2. Nucleotide sequences of various types of ITS1 of *P. carinii* f. sp. *hominis*. A sequence which is found in two or more specimens is considered a distinct type. A total of 15 ITS1 types, designated A through O, have been found. Two sequences representing each type are aligned. Bases that are identical to those of the consensus sequence are indicated by periods, missing bases are indicated by hyphens, and bases that are different from those of the consensus sequence are given. The sequences at positions 61 to 70 and 91 to 110 are not shown, because no sequence variations are found in these areas.

the most prevalent; 59 of 217 specimens contain this type. The second most prevalent type is Ne (43 specimens), which is followed by Eb (25 specimens); Ai (12 specimens); Bi, Ec, and Ee (11 specimens each); Al and Me (10 specimens each); Eh (7 specimens); Gg, Jf, and Kf (6 specimens each); Bl, Ei, and Og (4 specimens each); Bb, Bg, and Li (3 specimens each); Ad, Bk, De, Di, Ea, Ej, Em, Fg, Gb, Ih, Ng, Oe, and On (2 specimens each); and Ab, Ba, Be, Bh, Bm, Cg, Dh, Ed, Ef, El, Gf, Gh, Gi, He, Hg, Hh, Ie, Ii, In, Jg, Na, Nb, Nc, Nl, Nn, Of, and Oi (1 specimen each). Among the 207 specimens examined, 155 (75%) are found to contain a single type of *P. carinii* and 52 (25%) had more than one type, including 33 specimens with two types, 8 specimens with three types, 6 specimens with four types, and two specimens with 6 types (Table 3).

In this study, substantial numbers of specimens were collected from Denmark and the United States. A total of 45 types were found in the 123 specimens from Denmark. The three most prevalent types in Denmark are Eg (38 specimens), Ne (26 specimens), and Eb (15 specimens). Twenty-one different types were found in 44 specimens from the United States, and the three most prevalent types are Ne (13 specimens), Eg (11 specimens), and Eb (6 specimens). The numbers of specimens from other countries are not sufficient to make a significant conclusion on the prevalence of different types.

DISCUSSION

We have previously described the finding of four types of *P. carinii* based on nucleotide sequence variations in ITS regions, but there were a number of sporadic sequence variations which were not considered distinct types because less than three isolates had the same sequence (15). By expanding the sample size in this study, we have found more isolates that have the same sequence as those previously considered as sporadic variations. Most of those sporadic variations are now considered new types. We have also discovered many more new sequences.

Although a single nucleotide variation may result in a dis-

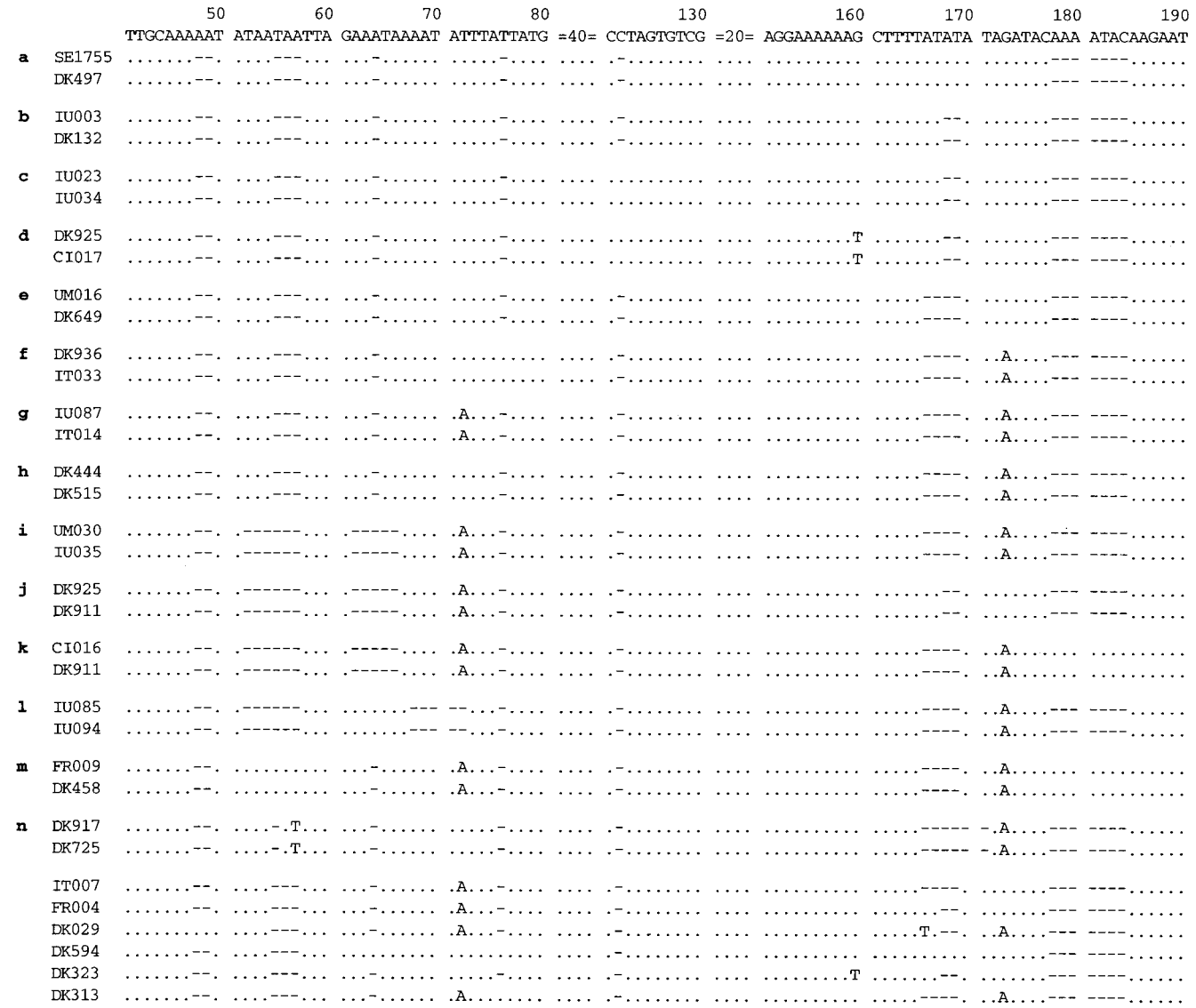

FIG. 3. Nucleotide sequences of various types of ITS2 of *P. carinii* f. sp. *hominis*. A sequence which is found in two or more specimens is considered a distinct type, and 14 sequences (designated a through n) met this requirement. Two sequences representing each type are aligned. Bases that are identical to those of the consensus sequence are indicated by periods, missing bases are indicated by hyphens, and bases that are different from those of the consensus sequence are given. The sequences at positions 1 to 40, 81 to 120, and 131 to 150, are not shown, because no sequence variations are found in these areas. Six sequences from specimens (IT007, FR004, DK029, DK594, DK323, and DK313) are also listed but are not considered distinct types, because they are found in only one specimen each.

tinct type, sequence variation in one area within ITS1 was not used for typing. This area is located at positions 62 to 71, where there are 10 T's in a row (Fig. 1). It has been reported that the number of T's determined in a poly(T) tract varied when the same sample was sequenced repeatedly (6, 20). In this study, the number of T's was found to vary from 8 to 12 in different specimens. Although there are several other poly(T) and poly(A) tracts in both ITS1 and ITS2, we did not encounter the same problem in these areas.

Tsolaki et al. (19) has recently examined 24 specimens and found five types of ITS1 (designated A_2, B_1, B_2, B_3, and C) and seven types of ITS2 sequences (designated a_1, a_2, a_3, b_1, b_2, c, and d). Types B_1, B_2, and C of Tsolaki et al. are the same as our new ITS1 types E, N, and F, respectively. Type A_2 can be type A or B and type B_3 can be type C or D, depending on the presence (types A and D) or absence (types B and C) of a T residue at position 12 of the ITS1 sequence. ITS2 types a_1, a_3, b_1, c, and d of Tsolaki et al. are the same as our new types e, g, b, l, and a, respectively. Type a_2 has the same sequence as that of IT007 (Fig. 3), which is not considered a distinct type in this study, because only one sample has this sequence. Type b_2 can be type c or d, depending on whether there is a G (type c) or T (type d) residue at position 160 of the ITS2 sequence.

Similar results have been reported by Latouche et al. (10, 11). They have examined 20 specimens in one study (10) and 36 in another (11) from AIDS patients and found eight types of ITS1 (designated A1, A2, B1, B2, B3, B4, B5, and B6) and seven types of ITS2 (designated a1, a2, a3, a4, b1, b5, and c1). ITS1 types A1 and A2 of Latouche et al. (11) are identical to our new ITS1 types B and A, respectively. Types B2 and B5

TABLE 2. *P. carinii* f. sp. *hominis* types found in various countries

Type no.	Type	No. of specimens found in country[a]:									Total no.[b]
		DK	US	CI	IT	FR	NE	PT	SE	TH	
1	Ab	1									1
2	Ad	1		1							2
3	Ai	5	2	2			3				12
4	Al	4	5					1			10
5	Ba	1									1
6	Bb	1	1			1					3
7	Be		1								1
8	Bg	3									3
9	Bh	1									1
10	Bi	6	2	2		1					11
11	Bk	1		1							2
12	Bl	2						2			4
13	Bm	1									1
14	Cg	1									1
15	De	1		1							2
16	Dh			1							1
17	Di	1								1	2
18	Ea	2									2
19	Eb	15	6			3		1			25
20	Ec	5	5	1							11
21	Ed	1									1
22	Ee	8	2	1							11
23	Ef	1									1
24	Eg	38	11	3	1	2			3	1	59
25	Eh	6	1								7
26	Ei	3				1					4
27	Ej	2									2
28	El	1									1
29	Em	1				1					2
30	Fg	1		1							2
31	Gb	1		1							2
32	Gf				1						1
33	Gg	3	2	1							6
34	Gh									1	1
35	Gi					1					1
36	He	1									1
37	Hg				1						1
38	Hh		1								1
39	Ie	1									1
40	Ih	1			1						2
41	Ii	1									1
42	In	1									1
43	Jf	5			1						6
44	Jg				1						1
45	Kf	1	1	1	3						6
46	Li		1	2							3
47	Me	4	2	4							10
48	Na								1		1
49	Nb		1								1
50	Nc		1								1
51	Ne	26	13	1		1	1		1		43
52	Ng	1	1								2
53	Nl	1									1
54	Nn	1									1
55	Oe		1	1							2
56	Of				1						1
57	Og	1	1	1					1		4
58	Oi						1				1
59	On	2									2
Total[c]		45	21	18	8	8	3	3	4	3	

[a] Abbreviations: DK, Denmark; US, United States; CI, Ivory Coast; IT, Italy; FR, France; NE, The Netherlands; PT, Portugal; SE, Sweden; TH, Thailand.
[b] Total number of each type found in this study.
[c] Total numbers of types in each country.

TABLE 3. Types of *P. carinii* f. sp. *hominis* found in each specimen

Sp. ID[a]	Type(s)	Sp. ID	Type(s)	Sp. ID[a]	Type(s)	Sp. ID[a]	Type(s)
DK004	Ne	DK412	Bg, Bi, Ne	DK713	Bl, Eg	IU085	Al
DK005	Eg	DK414	Jf	DK714	Bi, Ea, Eg	IU086	Al
DK006	Ee	DK415	Eg, Jf	DK720	Eg	IU087	Eg
DK008	Eg	DK439	Eb	DK725	Ii, In, On	IU088	Ne
DK010	Ne	DK441	Ng, Eh	DK735	Bg	IU089	Eg, Eh
DK013	Eg	DK444	Bi, Bg, Bh	DK911	Ai, Bi, Bk, Ed, Ej, Em	NJ114	Ne
DK015	Eg	DK451	Al, Nn	DK912	Eg	NJ00A	Ec
DK017	He, Me	DK458	Bi, Bm	DK913	Gg	NJ00W	Eg
DK027	Eg, Ef, Kf	DK464	Eb, Eg	DK918	Ee, Ei	NJ00Z	Eb
DK033	Ne	DK465	Eb, Eg	DK921	Eb	UM016	Eb, Nb, Ne
DK035	Oh	DK472	Ee	DK922	Ne	UM030	Ai
DK038	Ee, Me	DK478	Ai, Gg	DK923	Ne	CI006	Bi, Dh, Li
DK057	Eb, Ih	DK482	Eg	DK925	Ab, Ad, Eb, Ei, Ej, Gb, On	CI010	Ai, De, Gb, Oe
DK058	Eg	DK484	Eb	DK931	Ne	CI011	Ai
DK071	Og	DK488	Eg	DK937	Jf	CI012	Me, Ne
DK072	Ne	DK494	Ec, Eh	DK939	Ne	CI014	Bi, Ec, Eg, Li
DK073	Me	DK495	Cg, Eg	DK941	Ai	CI015	Ee, Gg, Me, Og
DK132	Eb	DK497	Ea	DK942	Ai	CI016	Bk
DK140	Ec	DK507	Eh	DK944	Eb	CI017	Ad
DK237	Bl	DK512	Eh, Ne	IU001	Ne	CI018	Eg
DK241	Nl	DK515	Eh	IU002	Bi	CI019	Eg, Fg, Kf
DK246	Eg	DK516	Eg, Ei	IU003	Bb, Bi, Eb	IT007	Eo
DK254	Eg	DK521	Ne	IU004	Eg	IT014	Eg
DK255	Ne	DK530	Eg	IU023	Ec, Nc, Ne, Oe	IT022	Hg
DK258	Jf	DK543	Ne	IU031	Ne	IT029	Kf
DK272	Ec	DK544	Eg	IU033	Ai	IT033	Gf, Of
DK275	Eg	DK545	Eg	IU034	Ec	IT040	Kf
DK284	Eb	DK547	Eg	IU035	Be, Eg, Li	IT046	Kf
DK288	Al	DK561	Ee	IU036	Ne	IT057	Ih
DK295	Ne	DK563	Ec	IU037	Ne	IT062	Jf, Jg
DK296	Eg	DK567	Ne	IU038	Ne	FR001	Eb
DK300	Eg	DK574	Ie	IU039	Eg	FR002	Ne
DK303	Ne	DK601	Eg	IU040	Kf	FR004	Eb, Eg, Eo
DK310	Ee	DK622	Ne, De	IU041	Hh	FR006	Bb, Eb, Eg, Ei
DK313	Eg	DK630	Eb	IU043	Og	FR009	Em
DK315	Eg	DK631	Eg	IU044	Eg, Gg	FR010	Bi, Gi
DK319	Eg	DK641	Eg	IU047	Ee	NL091	Ne
DK321	Gg	DK647	Eg	IU050	Eg	NL092	Ai
DK330	Eb	DK649	Ee	IU051	Ee, Eg, Ne, Ng	NL093	Ai
DK332	Jf	DK650	Eg	IU052	Gg	NL097	Ai, Oi
DK336	Al	DK651	Ba	IU058	Ec	PT115	Bl
DK338	Eb	DK652	Fg	IU059	Eg	PT153	Al
DK340	Eb	DK654	Ne	IU070	Ne	PT154	Bl
DK341	Ne	DK654-B	Ne	IU071	Ne	PT167	Eb
DK345	Ec	DK665	Ai, Di	IU077	Eb	SE911	Eg
DK346	Ne	DK669	Ne	IU078	Al, Eb	SE1337	Eg
DK347	Eh	DK671	Ne	IU079	Al, Me	SE1632	Ne, Og
DK375	Al	DK674	Bb	IU080	Me	SE1755	Na
DK399	Eg	DK676	El, Me	IU081	Ne	SE1969	Eg
DK402	Eg, Ne	DK681	Ne	IU082	Al	TH001	Di
DK403	Bi, Eg	DK698	Eb	IU083	Ec	TH002	Eg, Gh
DK406	Ne	DK702	Ee, Eg	IU084	Eb		

[a] Sp. ID, specimen identification.

differ in the numbers of T's in the poly(T) tract, which is not used for typing in our study. These two types should be considered the same and are equivalent to our type N. Type B3 is the same as our type J, except that position 21 is an A instead of a T. Similarly, type B4 is the same as our type I, except that position 21 is an A instead of a T. Types B1 and B6 of Latouche et al. (11) also differ in the numbers of T's in the poly(T) tract. These two types can be our type E, F, L, or M, depending on the nucleotides at positions 34, 42, and 115 to 118 (Fig. 2). Since the identities of nucleotides of types B1 and B6 at these positions are not reported, we are unable to identify these two types. In the ITS2 region, types a1, a2, a3, a4, b5, and c1 of Latouche et al. (11) are identical to our new types e, h, n, f, a, and l, respectively. Their type b1 can be our new type b, c, or d, depending on the nucleotides at positions 122 and 160. If the C residue at position 122 is missing, it is a type b. At position 160, type c has a G and type d has a T. Since there is no information as to the nucleotides at these two positions, it is impossible to determine whether their type b1 belongs to our type b, c, or d.

Among the 59 types of *P. carinii* f. sp. *hominis* found in this study, types Eg, Ne, and Eb are present in most countries from which we have obtained specimens. There are types that are found only in certain countries in this study. For example, types Be, Hh, Nb, and Nc are found only in the United States. However, it is premature to conclude that these types are unique to certain regions, since the sample size from each country was relatively small in this study. A considerably greater number of specimens from each country will have to be typed to make a significant conclusion. This study focused on finding new types rather than studying the distribution of different types.

As reported previously (2, 8, 9, 11, 15, 19), a portion of specimens examined in this study contained more than one type of *P. carinii*. Although it is likely that these specimens represent mixed infections, the following possibilities remain to be ruled out. The mixed types may be derived from different copies of rRNA genes in the same *P. carinii* genome, since multiple copies of rRNA genes are commonly found in eukaryotic organisms. It is unknown whether *P. carinii* f. sp. *hominis* has more than one copy of the rRNA gene, although rat-derived *P. carinii* has been shown to contain less than two copies (3). The other possibility is that they are due to heterozygosity of the organism. There is also the possibility of contamination during specimen collection or processing. In this study, two specimens were found to contain six or more types of *P. carinii*. It is quite likely that these two specimens were contaminated, since it is unusual to see a host infected by so many different types of the same organism.

Typing of *P. carinii* based on nucleotide sequence variations has been conducted by several investigators on several different loci (1, 5, 8–10, 12, 19, 20). We have examined the sequences of mitochondrial rRNA gene (12) and the ITS regions (7, 15, 16). In addition to these two loci, Latouche et al. (10) have examined the 5S rRNA gene and the thymidylate synthase gene. Sequence variations were not found in the thymidylate synthase gene. The mitochondrial rRNA gene has only 2 or 3 nucleotides that are variable. This limited sequence variation may not have sufficient discriminatory power for typing. Isolates that have the same mitochondrial rRNA gene type may have different ITS types (9). The 5S rRNA gene also has limited sequence variation; variations are found only in five positions. Until other genetic loci of *P. carinii* are found that are more variable, we consider ITS sequence variation the method of choice for typing *P. carinii* isolates. The development of *P. carinii* typing has enabled preliminary epidemiological studies (14) and investigation of potential air-borne transmission of *P. carinii* infections (2). It has also made it possible to determine whether recurrent pneumocystosis is due to a relapse of a previous infection or a new infection (8, 19).

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