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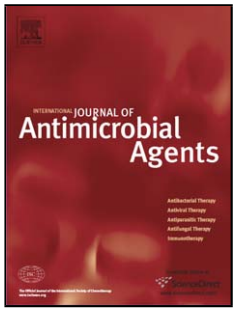

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PII: S0924-8579(09)00100-9
DOI: doi:10.1016/j.ijantimicag.2009.02.014
Reference: ANTAGE 2995

To appear in: *International Journal of Antimicrobial Agents*

Received date: 30-1-2009
Revised date: 11-2-2009
Accepted date: 12-2-2009

Please cite this article as: Fenollar F, Rolain J-M, Alric L, Papo T, Chauveheid M-P, van de Beek D, Raoult D, Resistance to trimethoprim/sulphamethoxazole and *Tropheryma whipplei*, *International Journal of Antimicrobial Agents* (2008), doi:10.1016/j.ijantimicag.2009.02.014

Resistance to trimethoprim/sulphamethoxazole and *Tropheryma whipplei*

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Received 30 January 2009; accepted 12 February 2009

Keywords: Whipple's disease; *Tropheryma whipplei*; Trimethoprim/sulphamethoxazole; Sulfadiazine; Antibiotic resistance

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Abstract

Whipple's disease (WD) is a chronic infection caused by *Tropheryma whipplei*. A 1-year treatment of oral trimethoprim/sulphamethoxazole (SXT) is commonly used. Advances in the culture of *T. whipplei* has allowed for full genome sequencing and antibiotic susceptibility testing, which has demonstrated resistance of *T. whipplei* to trimethoprim. Several mutations in the *folP* gene that encodes dihydropteroate synthase, the target of sulphonamides, has been reported for one patient with clinically acquired resistance to SXT. Here we report three new patients who experienced clinically acquired resistance to SXT during treatment and one patient with biological failure. Sixty-two *folP* sequences from DNA samples of 59 WD patients were also obtained. Among the detected amino acid changes, two positions (N4S and S234F) significantly predicted secondary sulphamethoxazole failure (four of five). We suggest that these mutations should be detected at the time of WD diagnosis by sequencing *folP* in order to avoid sulphamethoxazole monotherapy.

1. Introduction

Whipple's disease (WD) is a chronic infection caused by the bacterium *Tropheryma whipplei*, which was fatal before the advent of antibiotics [1]. Since 1952 and the first successful use of chloramphenicol, treatment has been empirical until the possible determination of antibiotic susceptibilities [1]. In 1966, Ruffin et al. [2] proposed a therapy based on penicillin and streptomycin administered parentally for 2 weeks, named induction therapy, followed by tetracycline orally for 3–12 months. Physicians then described patients who responded to numerous antibiotics, including tetracyclines, chloramphenicol, penicillin, streptomycin and trimethoprim/sulphamethoxazole (SXT). For many years, tetracycline was the drug of choice for maintenance therapy. In 1985, a study revealed a relapse rate as high as 35% among tetracycline-treated patients, with a high rate of central nervous system (CNS) relapse [3]. Induction treatment of parenteral streptomycin together with penicillin G for 2 weeks, followed by maintenance therapy of oral SXT [160 mg trimethoprim (TMP) and 800 mg (SMX) sulphamethoxazole] twice daily for 1 year was then proposed as a 'reasonable compromise' [4].

Currently, there is lack of an accurate definition of treatment failure because of misinterpretation of previous studies as well as a lack of follow-up to evaluate relapses. Primary failure after initiation of adequate WD therapy should be differentiated from relapse after antibiotic cessation (Fig. 1). Primary failure includes immediate failure observed <3 months after initiation of antibiotics and late failure observed >3 months after initiation of antibiotics. Immediate failure includes either a lack of amelioration, mainly described in patients with initial neurological involvement and treated with tetracyclines, and symptom exacerbation, which may be observed

during leprosy during lepromatous reactions or in immune reconstitution [5,6]. Such immediate failures may not be due to antibiotic failure or resistance. Late failure corresponds with acquired resistance to the treatment and is mainly described for SXT [7,8].

Owing to recent success in *T. whipplei* culture methods, susceptibility tests and full genome sequencing have been achieved. Many antibiotics, including doxycycline and SXT, are active in vitro [1]. Ceftriaxone and levofloxacin are active in axenic cultures, but cephalosporins (including ceftriaxone) and fluoroquinolones are not active in cell cultures. Genomic analysis indicates that *T. whipplei* lacks the coding sequence for dihydrofolate reductase, which is a target of TMP [5]. In vitro tests confirm that TMP is not active, whereas sulphonamide compounds, such as SMX and sulfadiazine, exhibit activity [5]. Current recommendations for WD treatment involve sulphonamide monotherapy. Recently, we reported mutations in the target gene of SMX, *folP*, that lead to in vitro resistance and secondary clinical failure [8]. Herein, we report three new patients with secondary clinical failure in response to SXT, one patient who developed biological failure as well as 62 complete sequences of *folP* from 59 patients.

2. Patients, materials and methods

2.1. Patient recruitment

Samples were sent from all over Europe for WD diagnosis [9]. Every time a result was consistent with WD, the physicians were asked to provide more data.

2.2. Definition of acquired resistance

Clinically acquired resistance to SXT is defined by the presence of two conditions: (i) an initial clinical response to oral SXT using the adequate dose (320 mg TMP/1600 mg SMX per day) described in the literature, with complete disappearance of clinical signs; and (ii) recurrence of the clinical symptoms observed at the time of WD diagnosis under the same treatment with SXT.

Biologically acquired resistance to SXT is defined by the presence of two conditions: (i) an initial clinical and biological response to oral SXT using the adequate dose, with complete disappearance of clinical signs and negative polymerase chain reaction (PCR) analysis; and (ii) reappearance of a positive PCR test under the same treatment with SXT.

2.3. PCR amplification and sequencing

Primers used to amplify and sequence *folP*, the gene encoding dihydropteroate synthase (DHPS), the target of SMX, and designed as described previously [8], are shown in Supplementary Table 1. The nucleotide and amino acid sequences obtained were compared as previously reported [8].

2.4. Statistical analysis

Statistical analyses were performed using Epi Info 6.0 software (http://www.cdc.gov/epiinfo/Epi6/EI6dnjp.htm). Differences were considered statistically significant at a *P*-value of <0.05.

3. Results

3.1. Clinical reports

Four of 44 patients from the database treated with SXT based on current recommendations developed clinically acquired resistance to SXT, and one patient presented a reappearance of a positive PCR in cerebrospinal fluid (CSF) (5/44; 11.4%).

3.1.1. Patient 1

A 78-year-old man was diagnosed with WD in 2003 [8]. Treatment with SXT resulted in the disappearance of manifestations. However, despite continual treatment, diarrhoea recurred 19 months later. *Tropheryma whipplei* PCR, performed as previously reported [9,10], was positive in duodenal tissue, saliva, stools, blood and CSF. Treatment with doxycycline (200 mg/day) and hydroxychloroquine [200 mg three times per day (tds)] was initiated. The patient quickly improved. Twenty-four months later, all the *T. whipplei* PCRs were negative. Periodic acid–Schiff (PAS) staining and specific immunohistochemistry, performed as previously reported [11], were slightly positive for duodenal biopsies. The patient is currently undergoing therapy. Mutations in the target gene of SMX have been previously detected in this patient [8].

3.1.2. Patient 2

A 71-year-old man was diagnosed with WD in 2001 based on weight loss, anorexia, asthenia, uveitis, positive PAS staining of duodenal biopsies and vitreous humour, and positive specific immunohistochemistry for duodenal biopsies, the vitreous

humour and blood samples. The patient was treated with SXT. Significant clinical improvement was initially observed. In March 2003 the patient began to present clinical manifestations, including asthenia, nausea, ataxia and oculofacial myorhythmia. The same treatment was administered. In January 2006, despite SXT treatment, the patient exhibited anorexia, weight loss and asthenia. *Tropheryma whipplei* PCR was positive for CSF and negative for blood. Doxycycline (200 mg/day) and hydroxychloroquine (200 mg tds) treatment was initiated. The patient presented significant clinical improvement. Eighteen months later, *T. whipplei* PCRs of saliva, stools, blood, CSF and duodenal biopsies were negative. PAS staining and specific immunohistochemistry were slightly positive for duodenal biopsies. The patient felt well and is still undergoing treatment.

3.1.3. Patient 3

A 70-year-old man was hospitalised in 2003 for fever, diarrhoea, weight loss and anaemia. WD was diagnosed in 1997 by positive PAS staining of duodenal biopsies. The patient was treated with SXT for 1 year. All symptoms disappeared. At the time of relapse, SXT treatment was started but did not lead to clinical improvement. PAS staining and specific immunohistochemistry were positive for duodenal biopsies. *Tropheryma whipplei* PCR was positive for duodenal biopsies and stool. Treatment comprised of doxycycline (200 mg/day), hydroxychloroquine (200 mg tds) and ceftriaxone (2 g/day) was initiated. Rapid improvement was observed. Ceftriaxone administration was then immediately stopped. The patient was treated for 20 months. Currently, 31 months after the end of treatment, the patient is doing well. *Tropheryma whipplei* PCRs of saliva, stools and blood are still negative.

3.1.4. Patient 4

A 61-year old man was diagnosed with WD in 2005 based on arthralgia, diarrhoea, weight loss and positive PAS staining of duodenal biopsies. Rapid disappearance of all symptoms was observed after starting SXT; however, after 10 months the patient presented new episodes of diarrhoea. Duodenal biopsies were still positive using PAS staining and specific immunohistochemistry. *Tropheryma whipplei* PCR was positive for duodenal biopsies and stool, and negative for saliva and CSF. Treatment consisting of doxycycline (200 mg/day) and hydroxychloroquine (200 mg tds) was initiated and rapid improvement was observed. After 19 months of treatment the patient remains well and is still receiving treatment.

3.1.5. Patient 5

A 62-year-old man was diagnosed with intestinal WD since 1985 on the basis of positive PAS staining of duodenal biopsies, which was first treated with SXT. In 2003 he had severe cognitive slowness and gait ataxia and was subsequently diagnosed with cerebral involvement of WD on the basis of a positive CSF *T. whipplei* PCR. He was treated with a 4-week course of intravenous ceftriaxone. He showed excellent recovery afterwards and had a negative PCR in the CSF. In 2004 he relapsed with headache, cognitive slowness on neuropsychological testing and positive CSF *T. whipplei* PCR. Subsequently, he was treated with SXT, again with excellent clinical recovery and negative CSF PCR. He continued with SXT afterwards. However, due to liver problems, SXT was brought down to half doses in 2007. Six months later he had another relapse with headache, subjective cognitive slowness and positive CSF *T. whipplei* PCR. He was treated with ceftriaxone (2 weeks) followed by SXT (320 mg TMP/1600 mg SMX per day). The symptoms disappeared, but *T. whipplei* PCRs

remained positive 6 months after ceftriaxone treatment, and CSF pleocytosis increased under SXT maintenance therapy.

3.2. PCR amplification and sequencing

Forty-one new amino acid *folP* sequences from 39 patients with *T. whipplei* infections have been obtained since our first report [8]. Among these 39 patients, 3 presented secondary clinical failure with SXT and one presented biological failure with the reappearance of positive *T. whipplei* PCR of CSF with SXT. For two of those patients with clinical failure, samples were available before and after clinical failure. For the third, a sample was available only after clinical failure. For the patient who presented a reappearance of positive PCR on CSF, only the specimen sampled after the biological failure was available. For the remaining 35 patients, they were either not treated with SXT at the time of sampling or information pertaining to therapy or follow-up were not known. Twenty-one amino acid sequences of DHPS previously described by Bakkali et al. [8] were added for comparison, including two from a patient with clinical failure during treatment with SXT and nineteen from laboratory strains from patients without any evidence of clinical failure or relapse.

Overall, 62 sequences from 59 patients were analysed. Patient details are summarised in Supplementary Table 2. Eight different amino acid sequence types were found and specific amino acid substitutions in the DHPS protein are shown in Tables 1 and 2. Amino acid sequences for all patients and samples before treatment are given in Table 1. Among the amino acid changes present at the time of diagnosis, two positions, i.e. N4S and S234F, were significantly associated with patients with failure. The N4S change was detected in 3 of 4 patients with failure and

in 3 of 54 patients without evidence of failure or without follow-up; this difference was significant (P = 0.0003). Moreover, the sensitivity of the N4S substitution to predict resistance before treatment was 50%, with 98% specificity, a positive predictive value (PPV) of 75% and a negative predictive value (NPV) of 94.4%. The S234F mutation was detected in 2 of 4 patients with failure and in 1 of 54 patients without evidence of failure or without follow-up; this difference was also significant (P = 0.0024). The sensitivity of the S234F change to predict failure before treatment was 66.7%, with a specificity of 96.4%, PPV of 50% and a NPV of 98.2%. If we focus on the sequences from patients at the time of failure, four (80%) of five presented a signature amino acid sequence with either N4S or S234F mutations (Table 2). Moreover, new mutations appeared in two out of three amino acid sequences with previous N4S or S234F changes.

4. Discussion

The literature is confusing with regard to evaluating WD treatment. Such confusion is mainly linked to the fact that current recommendations are considered established facts, although they are still based on successive deductive opinions. A lack of an accurate definition of treatment failure led to misinterpretation of previous studies. Follow-up was often too short to analyse relapses occurrence. Overall, 9.1% of our patients treated with SXT relapsed clinically during treatment. To the best of our knowledge, only three patients who experienced apparent acquired resistance clinically using SXT have been previously reported in the literature [6,7,12]. Moreover, data analysis on relapses of patients treated with SXT from several studies shows a low rate of 3%, which is probably grossly underestimated [3,6,13].

Recently, from data of the Study for the Initial treatment of Morbus Whipple (SIMW) among 40 WD patients treated with SXT (320 mg TMP/1600 mg SMX per day) over 1 year following a previous treatment with either 2 g ceftriaxone or three doses of 1 g meropenem each for 14 days intravenously, there was no relapse 3 years after completion of treatment [14]. However, since these data have not been published as a full manuscript but only as an abstract form, more comprehensive information regarding patients and methodologies is needed.

SXT was selected as a first-line regimen owing to the occurrence of neurological relapse in WD and the superior penetration of this compound into the CSF [3,6]. However, after excluding patients diagnosed with initial neurological involvement, the failure rate was the same in patients treated with tetracyclines or SXT [6]. In addition, when considering patients who initially presented with a CNS infection, conflicting results were observed. One study suggested that SXT was better than tetracyclines [6]. Another reported that 12 patients (40%) treated with SXT did not respond, clearly showing that this drug did not efficiently prevent neurological involvement [12].

Currently, SXT is a sulphonamide monotherapy in WD. As for WD, sulphonamides were shown early in the 20th century to be prone to rapid development of resistance [15]. Clinical failure, in vitro resistance to SMX and mutations of DHPS have been previously reported in a patient [8]. Herein, we show that the presence of two changes in the amino acid sequences of DHPS (N4S and/or S234F) detected at the time of diagnosis significantly predicted secondary clinical failure. Besides, new amino acid mutations appeared after treatment with SXT in sequences with previous

changes at the time of the diagnosis. The risk of resistance appears to be high with SMX monotherapy in patients with these mutations.

At the time of WD diagnosis, we suggest that the *folP* gene must be amplified and sequenced from a specimen positive for *T. whipplei* by PCR in order to detect the presence of mutations. This strategy may allow the early detection of resistance or the risk to develop resistance to sulphonamide drugs.

Acknowledgments: The authors wish to thank Julien Soupault for technical help.

Funding source: This work was supported by the French Centre National de la Recherche Scientifique (CNRS).

Competing interests: None declared.

Ethical approval: The present study was approved by the local ethics committee (agreement No. 07-036).

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Fig. 1. Schematic representation of failures and relapses in *Tropheryma whipplei* infections.

Table 1

Amino acid sequences of dihydropteroate synthase (DHPS) from 58 samples obtained from 58 patients at the time of diagnosis with *Tropheryma whipplei* infection but before treatment

Patient	Code	Sample	Outcome	Amino acid sequences of DHPS									
				4	42	57	102	162	203	225	232	234	246
1	FrDDbBTF1	DNA from Patient 1	Failure	S	T	V	T	L	N	S	N	S	N
2	FrDAhBTF2	DNA from Patient 2	Failure	S	T	V	T	L	N	S	D	F	N
3	FrDStATF3	DNA from Patient 3 [a]	Failure	N	T	V	T	L	N	S	D	S	N
4	ItDDbBTF4	DNA from Patient 4	Failure	S	T	V	T	L	N	S	D	F	N
5	FrDAdp	DNA	Other	N	T	V	T	L	N	S	D	S	N
6	FrDBl1	DNA	Other	N	T	V	T	L	N	S	D	S	N
7	FrDBl2	DNA	Other	N	T	V	T	L	N	S	D	S	N
8	FrDBl3	DNA	Other	N	T	V	T	L	N	S	D	S	N
9	FrDCsf	DNA	Other	N	T	V	T	L	N	S	D	S	N
10	FrDDb1	DNA	Other	N	T	V	T	L	N	S	D	S	N
11	FrDDb2	DNA	Other	N	T	V	T	L	N	S	D	S	N
12	FrDDb3	DNA	Other	N	T	V	T	L	N	S	D	S	N
13	FrDDb4	DNA	Other	N	T	V	T	L	N	S	D	S	N
14	FrDDb5	DNA	Other	S	T	V	T	L	S	S	N	S	N
15	FrDDb6	DNA	Other	N	T	V	T	L	N	S	D	S	N

16	FrDDb7	DNA	Other	N	T	V	T	L	N	S	D	S	N
17	FrDDb8	DNA	Other	N	T	V	T	L	N	S	D	S	N
18	FrDDb9	DNA	Other	N	T	V	T	L	N	S	D	S	N
19	FrDDb10	DNA	Other	N	T	V	T	L	N	S	D	S	N
20	FrDGb1	DNA	Other	S	T	V	T	L	N	S	D	F	N
21	FrDGb2	DNA	Other	N	T	V	T	L	N	S	D	S	N
22	FrDMus1	DNA	Other	N	T	V	T	L	N	S	D	S	N
23	FrDMus2	DNA	Other	N	T	V	T	L	N	S	D	S	N
24	FrDSal1	DNA	Other	N	T	V	T	L	N	S	D	S	N
25	FrDSal2	DNA	Other	N	T	V	T	L	N	S	D	S	N
26	FrDSal3	DNA	Other	N	T	V	T	L	N	S	D	S	N
27	FrDSal4	DNA	Other	N	T	V	T	L	N	S	D	S	N
28	FrDSt1	DNA	Other	N	T	V	T	L	N	S	D	S	N
29	FrDSt2	DNA	Other	N	T	V	T	L	N	S	D	S	N
30	FrDSt3	DNA	Other	N	T	V	T	L	N	S	D	S	N
31	FrECv	DNA	Other	N	T	V	T	L	N	S	D	S	N
32	FrPPb	DNA	Other	N	T	V	T	L	N	S	D	S	N
33	FrUAh	DNA	Other	N	T	V	T	L	N	S	D	S	N
34	ItDSt	DNA	Other	S	A	V	T	L	S	S	D	S	N
35	ItDCsf	DNA	Other	N	T	V	T	L	N	C	D	S	N
36	PortDDb	DNA	Other	N	T	V	T	L	N	S	D	S	I

37	PortECv	DNA	Other	N	T	V	T	L	N	S	D	S	N
38	SwitECv	DNA	Other	N	T	V	T	L	N	S	D	S	N
39	TunDGb	DNA	Other	N	T	V	T	L	N	S	D	S	N
40	TWIST	Strain	Other	N	T	V	T	L	N	S	D	S	N
41	ENDO 5	Strain	Other	N	T	V	T	L	N	S	D	S	N
42	NEURO 2	Strain	Other	N	T	V	T	L	N	S	D	S	N
43	DIG 7	Strain	Other	N	T	V	T	L	N	S	D	S	N
44	DIG 15	Strain	Other	N	T	V	T	L	N	S	D	S	N
45	SLOW 1B	Strain	Other	N	T	V	T	L	N	S	D	S	N
46	NEURO 1	Strain	Other	N	T	V	T	L	N	S	D	S	N
47	ENDO 7Bb	Strain	Other	N	T	V	T	L	N	S	D	S	N
48	DIG 9	Strain	Other	N	T	V	T	L	N	S	D	S	N
49	DIG 10	Strain	Other	N	T	V	T	L	N	S	D	S	N
50	DIG ADP 11	Strain	Other	N	T	V	T	L	N	S	D	S	N
51	ART 1	Strain	Other	N	T	V	T	L	N	S	D	S	N
52	DIG-NEURO 14	Strain	Other	N	T	V	T	L	N	S	D	S	N
53	DIG ADP 16	Strain	Other	N	T	V	T	L	N	S	D	S	N
54	DIG NEURO 18	Strain	Other	N	T	V	T	L	N	S	D	S	N
55	ENDO 19	Strain	Other	N	T	V	T	L	N	S	D	S	N
56	NEURO 21	Strain	Other	N	T	V	T	L	N	S	D	S	N
57	DIG NEURO 23	Strain	Other	N	T	V	T	L	N	S	D	S	N

58	TW08-27	Strain	Other	N	T	V	T	L	N	S	D	S	N

Fr, France; It, Italy; Port, Portugal; Swit, Switzerland; Tun, Tunisia; D, digestive Whipple's disease; E, endocarditis due to *T. whipplei*; P, pulmonary infection due to *T. whipplei*; U, uveitis due to *T. whipplei*; Db, duodenal biopsy; Ah, aqueous humour; St, stool; Adp, adenopathy; Bl, blood; Csf, cerebrospinal fluid; Gb, gastric biopsy; Mus, muscle; Sal, saliva; Cv, cardiac valve; Pb, pulmonary biopsy; BTF, before treatment failure; ATF, after treatment failure.

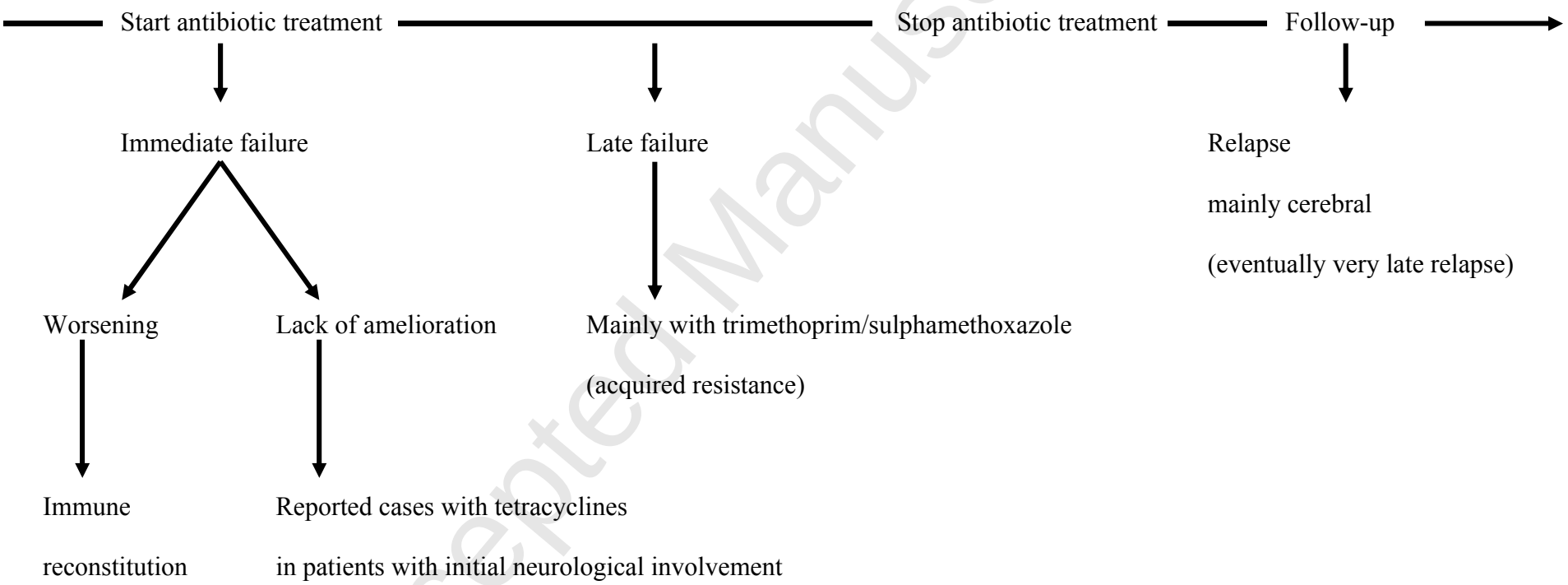
[a] No DNA from specimens sampled before the start of treatment was available for this patient. Thus, the amino acid sequence used in this table was obtained at the time of clinical failure. Indeed, we can suspect that both sequences (before and after failure) are the same as no mutation was detected in the sequence obtained at the time of the failure.

Table 2

Amino acid sequences of dihydropteroate synthase (DHPS) obtained from eight samples from five patients, including four patients with acquired clinical resistance (Patients 1, 2, 3 and 4) and one patient who presented reappearance of a positive polymerase chain reaction (PCR) from cerebrospinal fluid

Patient	Code	Sample	Amino acid sequences of DHPS									
			4	42	57	102	162	203	225	232	234	246
1	FrDDbBTF1	DNA	S	T	V	T	L	N	S	N	S	N
	FrDDbATF1	DNA	S	T	I	P	I	N	S	N	S	N
2	FrDAhBTF2	DNA	S	T	V	T	L	N	S	D	F	N
	FrDCsfATF2	DNA	S	T	V	T	L	S	S	D	F	N
3	FrDStATF3	DNA	N	T	V	T	L	N	S	D	S	N
4	ItDDbBTF4	DNA	S	T	V	T	L	N	S	D	F	N
	ItDDbATF4	DNA	S	T	V	T	L	N	S	D	F	N
5	NetDCsfATF	DNA	S	T	V	T	L	N	S	D	F	N

Fr, France; It, Italy; Net, Netherlands; D, digestive Whipple's disease; Db, duodenal biopsy; Ah, aqueous humor; Csf, cerebrospinal fluid; St, stool; BTF, before treatment failure; ATF, after treatment failure.



Supplementary Table 1

Primers used in this study to frame and amplify the *folP* sequences

Primer	Sequence
FolpF1	5'-ACC-TGC-ATT-TTG-GAT-GAA-GC-3'
FolpR1	5'-TAA-TGG-CAA-CAC-CCA-TGC-TA-3'
FolpF2	5'-CCC-ATT-TCC-ACC-TCC-TAA-TG-3'
FolpR2	5'-CGG-CAG-TAA-TAC-GCT-CGA-TA-3'
FolpF3	5'-GCC-TTG-CTC-ATT-ACG-ACG-AT-3'
FolpR3	5'-GTG-TGA-GCT-GAG-GCA-ACA-TC-3'

Supplementary Table 2

Details of the 59 patients included in the study

Patient code	Sex/age (years)	Main manifestations [a]	Main samples tested positive for *Tropheryma whipplei* [b]
FrDDbBTF1	M/78	A, diarrhoea	Db, Sal, St, Bl, Csf
FrDAhBTF2	M/71	WL, uveitis	Db, Bl, Ah, Csf
FrDStATF3	M/70	Diarrhoea, WL, fever	Db, St
ItDDbBTF4	M/61	A, diarrhoea, WL	Db, St
NetDCsfATF	M/62	Diarrhoea, neurological signs	Db, CSF
FrDAdp	F/57	A, WL, Adp, neurological signs	Db, Sal, St, Bl, Adp, Csf
FrDBl1	M/42	A, diarrhoea	Db, Sal, Bl
FrDBl2	M/60	A, diarrhoea, WL, AP, fever	Db, Bl
FrDBl3	M/58	A, diarrhoea, WL, AP, fever	Db, Bl
FrDCsf	F/57	A, Adp	Db, Adp
FrDDb1	F/58	A	Db, St, Bl
FrDDb2	M/77	Diarrhoea, Adp	Db, Adp
FrDDb3	M/66	Diarrhoea	Db, Sal, Bl
FrDDb4	M/46	A, fever	Db, Csf
FrDDb5	M/58	A, diarrhoea	Db, Sal, St
FrDDb6	M/60	A, WL, fever, Adp	Db, Sal, St
FrDDb7	M/35	A, WL, fever	Db

FrDDb8	F/69	A	Db
FrDDb9	M/56	A, diarrhoea	Db, Sal, St, Bl
FrDDb10	M/46	A	Db, Sal, Bl
FrDGb1	M/50	Diarrhoea	Db
FrDGb2	M/60	A, diarrhoea, Adp	Db, Sal, St, Bl
FrDMus1	F/73	A, WL, myalgia, Adp	Db, Sal
FrDMus2	M/67	A, fever, myalgia	Db, Bl
FrDSal1	F/71	Diarrhoea, WL, headache	Db, Sal, St
FrDSal2	M/74	A, diarrhoea, Adp	Db
FrDSal3	M/42	A, diarrhoea	Db, Sal, St, Csf
FrDSal4	M/43	A, diarrhoea, neurological signs	Db, Sal, Bl, Csf
FrDSt1	F/40	A, Adp, fever	Db, Sal, St, Bl
FrDSt2	M/40	A, diarrhoea, WL, AP, Adp, fever	Db, Sal, St
FrDSt3	M/50	A, diarrhoea, WL, AP	Db
FrECv	M/57	A, WL, fever, endocarditis, neurological signs	Db, Sal, St, Cv
FrPPb	F/55	Pulmonary signs	Sal, St, lung biopsy
FrUAh	F/78	Uveitis	Ah
ItDSt	M/NA	Diarrhoea	Db, St
ItDCsf	M/58	Diarrhoea, neurological signs	Db, Csf
PortDDb	M/50	Fever	Db, Sal, St

PortECv	M/58	Endocarditis	Cv
SwitECv	M/75	Endocarditis	Cv
TunDGb	M/NA	NA	Db
TWIST	M/42	Endocarditis	Cv
ENDO 5	M/61	Endocarditis	Bl, Cv
NEURO 2	F/40	A, neurological signs	Db, Sal, St, Csf
DIG 7	M/64	A, WL	Db, Sal, St, Bl
DIG 15	M/60	A, diarrhoea, WL, fever	Db, Csf
SLOW 1B	F/34	WL, AP	Db, St
NEURO 1	M/57	Diarrhoea, WL, neurological signs	Db, Csf
ENDO 7Bb	M/67	Endocarditis	Bl, Cv
DIG 9	M/35	A, diarrhoea, WL, fever, Adp	Db, Sal, St, Bl
DIG 10	M/46	A, diarrhoea, WL, neurological signs	Db, Csf, Adp
DIG ADP 11	M/56	Diarrhoea, WL	Db, St, Adp
ART 1	M/68	A	Db, Sf
DIG-NEURO 14	M/50	NA	Db, Csf
DIG ADP 16	F/38	A, diarrhoea, WL, AP, Adp	Db, Bl, Adp
DIG NEURO 18	M/68	A, Adp, fever, neurological signs	Db, St, Csf
ENDO 19	M/NA	Endocarditis	Cv

NEURO 21	M/57	Diarrhoea, pigmentation	Db, Csf
DIG NEURO 23	F/57	A, diarrhoea, AP, Adp, fever	Db, Sal, St, Csf
TW08-27	F/NA	NA	Csf

[a] A, arthralgia; WL, weight loss; Adp, adenopathy; AP, abdominal pain; NA, not available.

[b] Db, duodenal biopsy; Sal, saliva; St, stools; Bl, blood; Csf, cerebrospinal fluid; Ah, aqueous humour; Adp, adenopathy; Cv, cardiac valve; Sf, synovial fluid.