Article available at http://www.parasite-journal.org or http://dx.doi.org/10.1051/parasite/2010173219

# EPIDEMIOLOGY AND CLINICAL RELEVANCE OF *PNEUMOCYSTIS JIROVECII* FRENKEL, 1976 DIHYDROPTEROATE SYNTHASE GENE MUTATIONS[1]

MATOS O.* & ESTEVES F.*

**Summary:**

A review was conducted to examine the published works that studied the prevalence of *Pneumocystis jirovecii* dihydropteroate synthase (DHPS) mutations in patients with *P. jirovecii* pneumonia (PcP), in develop and developing countries, and that focused the problem of the possible association of these mutations with exposure to sulpha or sulphone drugs and their influence in the PcP outcome. Studies conducted in United States of America presented higher *P. jirovecii* mutations rates, in comparison with European countries, and in developing countries, lower rates of DHPS mutations were reported, due to limited use of sulpha drugs. A significant association was reported between the use of sulpha or sulphone agents for PcP prophylaxis in HIV-infected patients and the presence of DHPS mutations. However these mutations were also detected in PcP patients who were not currently receiving sulpha or sulphone agents. The outcome and mortality of HIV-infected patients with PcP harbouring DHPS gene mutations were related primarily to the underlying severity of illness and the initial severity of PcP, more than to the presence of mutations.

**KEY WORDS :** Pneumocystis jirovecii, dihydropteroate synthase, gene mutation, pneumonia, sulpha, sulphone, HIV.

***Résumé :*** ÉPIDÉMIOLOGIE ET PERTINENCE CLINIQUE DES MUTATIONS GÉNÉTIQUES DE LA DIHYDROPTEROATE SYNTHÉTASE CHEZ *PNEUMOCYSTIS JIROVECII* FRENKEL, 1976

*Une revue de la littérature a été conduite sur les travaux qui ont étudié la fréquence des mutations de la dihydropteroate synthétase (DHPS) de* Pneumocystis jirovecii *chez des patients atteints de pneumonies (PcP) provoquées par cet opportuniste, dans des pays développés et en voie de développement. Elle s'est focalisée sur le problème du possible lien de causalité de ces mutations avec l'exposition aux sulfamides et aux sulfones et l'influence sur leur efficacité dans le traitement des PcP. Les études conduites aux États-Unis ont montré les taux de mutation de la DHPS les plus élevés en comparaison de ceux observés dans des pays européens. Dans les pays en voie de développement, les taux bas de mutations ont été rapportés à l'utilisation limitée de sulfamides. Une association significative a été observée entre l'utilisation de sulfamides et de sulfones dans la prophylaxie des PcP chez des patients infectés par le VIH et la présence de mutations. Cependant des mutations ont aussi été détectées chez des patients atteints de PcP qui n'avaient pas reçu de sulfamides ou de sulfones. Le devenir et la mortalité de patients infectés par le VIH et atteints de PcP avec mutation de la DHPS sont liés principalement à la sévérité de l'infection virale sous-jacente et la sévérité initiale de la PcP, plus qu'à la présence d'une mutation.*

***MOTS CLÉS :*** Pneumocystis jirovecii, *dihydropteroate synthétase, mutation génétique, pneumonie, sulfamide, sulfone, VIH.*

## INTRODUCTION

*Pneumocystis jirovecii* (formerly *Pneumocystis carinii* f. sp. *hominis*) (Stringer *et al.*, 2002; Redhead *et al.*, 2006) is recognized as a worldwide pathogen that causes severe interstitial pneumonia (PcP) in immunodeficient patients, especially in the human immunodeficiency virus (HIV) patients (Wakefield, 2002). Widespread use of PcP chemoprophylaxis and Highly Active Antiretroviral Therapy (HAART) have reduced the incidence of this pathology in developed countries (Palella *et al.*, 1998; Barry & Johnson, 2001; Morris *et al.*, 2004). Despite these advances, in the XXI century, PcP continues to be a serious problem for HIV-infected patients, especially for those not previously known to be seropositive for the virus (Kaplan *et al.*, 2000); for patients non-adherent to treatment, particularly those with a history of injecting drug use, and those with active substance abuse or psychiatric illness; for those who are not receiving or responding to antiretroviral therapy or prophylaxis because of factors related to pharmacokinetics, or unexplained biologic factors (Lundberg *et al.*, 2000; Kaplan *et al.*, 2009); or those with advanced immunodeficiency ($CD4^+$ T cells count < 100 cells/microliter [µL] blood), (Wolff & O'Donnell, 2001). *P. jirovecii* has also been isolated from non-HIV-infected immunodeficient patients, such as cancer patients and transplant recipients, and more recently from patients with rheumatologic pathologies and with

* Unidade de Protozoários Oportunistas/VIH e Outras Protozooses, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisboa, Portugal.
Correspondence: Olga Matos
Tel.: +351 213 652638 – Fax: +351 213 632105.
E-mail: omatos@ihmt.unl.pt.

[1] Stemming text to a communication at the "First centenary of the *Pneumocystis* discovery", Brussels, 5-6 November 2009.

inflammatory bowel disease (Sepkowitz 2002; Kaneko *et al.*, 2006; Poppers & Scherl, 2008). This organism has also been isolated from immunocompetent persons and also patients with lung tissue damage caused by different clinical conditions are at increased risk of *P. jirovecii* colonization (Pifer *et al.*, 1978; Matos *et al.*, 2003; Montes-Cano *et al.*, 2004; Medrano *et al.*, 2005; Matos *et al.*, 2006; Vidal *et al.*, 2006; Esteves *et al.*, 2008). Also, in developing regions of the Globe, where most HIV-infected persons reside, there is an increased incidence of PcP since the access to HAART and PcP prophylaxis is still limited (WHO, 2003; French *et al.*, 2006). Therefore, prevention of PcP cases continues to be an important goal. Lifelong PcP prophylaxis is recommended for all HIV-infected patients with less than 200 $CD4^+$ T-cells/µL (Kaplan *et al.*, 2002).

Sulpha and sulphone drugs are key prophylactic and treatment agents for PcP (CDC 1992; Bozzette *et al.*, 1995). The antimicrobial combination of trimethoprim and sulfamethoxazole (TMP-SMZ) has been the first line drug for treatment and prophylaxis of PcP, for almost three decades. Another sulfone agent, dapsone, is an important second-line prophylactic drug against *P. jirovecii* (Kaplan *et al.*, 2002). Since sulpha resistance has developed in an increased number of common bacteria isolated in HIV-infected and non-HIV-infected patients, coinciding with the rise in TMP-SMZ prophylaxis for PcP (Martin *et al.*, 1999), it looked plausible that *P. jirovecii* could become resistant to sulpha and sulphone agents. Because this organism cannot be cultivated *in vitro* determination of resistance by traditional methods is not possible. The recent application of PCR-based molecular methods have detected mutations in *P. jirovecii* DHPS. Subsequent reports showed that dihydropteroate synthase (DHPS) mutations were associated with failures of sulpha and sulphone prophylactic drugs in acquired immunodeficiency syndrome (AIDS) patients with PcP (Kazanjian *et al.*, 1998).

## MECHANISM OF ACTION OF SULPHONAMIDE AGENTS

Unlike the vertebrate host that exogenously acquired folic acid, *P. jirovecii* is unable to scavenge folates from the environment, being absolutely dependent on its own mechanism for de novo synthesis of this molecule (Kovacs *et al.*, 1989). Folic acid is required in several metabolic pathways, including the synthesis of purines, thymidine, glycine and methionine. Thus, inhibition of enzymes connected to the synthesis of folic acid, leads *P. jirovecii* cells to collapse for the lack of amino acids for protein synthesis and for the limited amount of nucleotide bases for DNA repair, replication and transcription (Armstrong *et al.*, 2000). The initial enzyme in de novo folate synthesis, DHPS, is part of a trifunctional protein along with two other enzymes in folic acid biosynthesis pathway, dihydroneopterin aldolase and hydroxymethyldihydropterin pyrophosphokinase (Volpe *et al.*, 1992). DHPS catalyses the condensation of para-aminobenzoic acid (PABA) with 6-hydroxymethyl-7,8-dihydropterin pyrophosphate (DHPPP), forming 7,8-dihydropteroate, that is subsequently converted into 7,8-dihydrofolate by the enzyme dihydrofolate synthase (Fig. 1). Dihydrofolate reductase (DHFR) catalyses the reduction of 7,8-dihydrofolate to tetrahydrofolate and is dependent on the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) (Ma *et al.*, 1999; Armstrong *et al.*, 2000). Folate

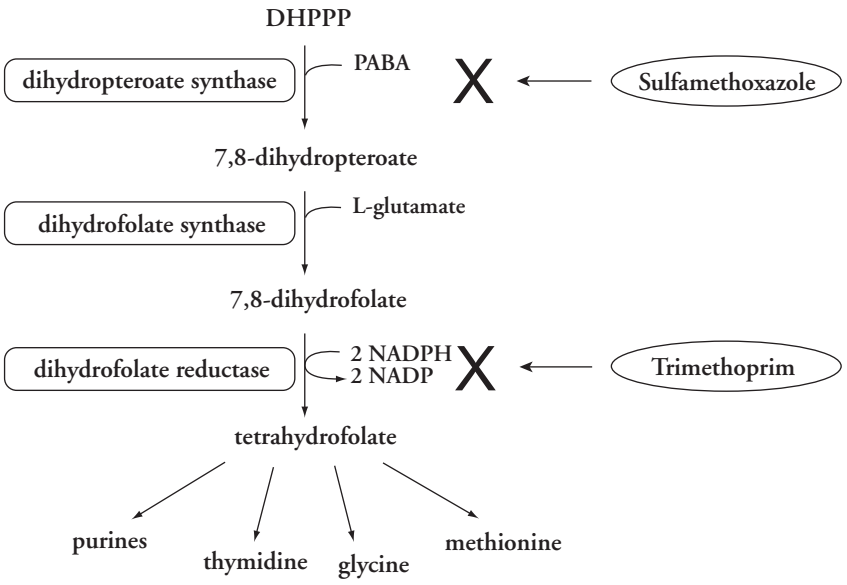


Fig. 1. – The folic acid synthesis pathway and TMP-SMZ action points.

antagonists, including trimethoprim (TMP) and sulfamethoxazole (SMZ), are known to be active against *P. jirovecii*. The drug combination TMP-SMZ has been implemented for treatment and prevention of PcP. This synergic combination of two molecules, members of the class of folate antagonist drugs, has been proven effective against *P. jirovecii* and is currently the recommended first-line therapy for the treatment of PcP (Masur, 1992; Klein *et al.*, 1992; Barry & Johnson, 2001; Benson *et al.*, 2004). Whereas trimethoprim inhibits DHFR, sulphamethoxazole, like other sulpha and sulphone agents, is a PABA analogue that targets DHPS acting as a competitive inhibitor (Armstrong *et al.* 2000).

## DHPS MUTATIONS IN *P. JIROVECII*

The widespread use of inhibitors of folate metabolism in antimicrobial chemotherapy has resulted in the emergence of specific drug resistance in several organisms (Lane *et al.*, 1997; Ma *et al.*, 1999; Armstrong *et al.*, 2000; Huang *et al.*, 2004). The standard anti-*P. jirovecii* chemotherapy with the combination TMP-SMX attempt to block the folate metabolic pathway at two sequential points, producing a synergistic pharmacological effect that has been demonstrated *in vitro* to be active against several microorganisms (Bushby, 1983; Armstrong *et al.*, 2000; Huang *et al.*, 2004). However, in the rat model, the effect of TMP-SMZ is almost entirely attributed to sulfamethoxazole (Walzer *et al.*, 1992). The molecular mechanisms of antifolate resistance, particularly in the DHPS locus, have been studied extensively in numerous bacteria and some protozoa, like *Plasmodium falciparum*, whereas DHPS point mutations are associated with sulpha resistance (Brooks *et al.*, 1994; Triglia *et al.*, 1998). Recently, mutant sequences of *P. jirovecii* DHPS gene have been detected. Several point mutations have been identified in patients receiving anti-*P. jirovecii* prophylaxis with TMP-SMZ or dapsone, suggesting that point mutations in DHPS gene may be a consequence of exposure to sulpha drugs and that the widespread use of TMP-SMZ may be exerting a selective pressure of *P. jirovecii* genotypes circulating among humans (Lane *et al.*, 1997; Ma *et al.*, 1999; Huang *et al.*, 2000). Other studies have associated the occurrence of specific mutations in the *P. jirovecii* DHPS gene with the failure of sulpha or sulphone prophylaxis and with the outcome HIV-associated PcP, which suggests the emergence of sulpha or sulphone-resistant strains of *P. jirovecii* (Kazanjian *et al.*, 1998; Mei *et al.*, 1998; Helweg-Larsen *et al.*, 1999; Kazanjian *et al.*, 2000; Takahashi *et al.*, 2000; Visconti *et al.*, 2001; Crothers *et al.*, 2005). Therefore, several *P. jirovecii* DHPS point mutations have been studied and are suspected of association with TMP-SMZ resistance. Whether these mutations confer resistance to sulpha or sulphone agents in PcP treatment remains unclear (Huang *et al.*, 2004). Table I summarizes all the *P. jirovecii* DHPS point mutations described so far. Some of these mutations were detected in highly conserved regions of the enzyme. The amino acids Phe$^{23}$, Thr$^{55}$, Pro$^{57}$, Ile$^{75}$, Leu$^{78}$, Ala$^{100}$ and Ile$^{111}$ occur in regions of the protein that are conserved among *P. jirovecii* and rat and mouse derived *P. carinii*. The

| ID | Nucleotide (position : identity) | Amino acid (position : identity) | Codon | Exposure to sulpha / sulphone agent | Reference (first time described) | Access numbers (GeneBank) |
|---|---|---|---|---|---|---|
| 23 | 71 : **C**/G | 23 : **Phe**/Leu | **TTC**/TTG | No | Lane *et al.*, 1997 | PCU66280 |
| 55 | 165 : **A**/G | 55 : **Thr**/Ala | **ACA**/GCA | Yes | Lane *et al.*, 1997 | PCU66281 |
| 57 | 171 : **C**/T | 57 : **Pro**/Ser | **CCT**/TCT | Yes | Lane *et al.*, 1997 | PCU66278 and PCU66281 |
| 60 | 180 : **C**/G | 60 : **His**/Asp | **CAT**/GAT | No | Lane *et al.*, 1997 | PCU66280 |
| 75 | 226 : **T**/C | 75 : **Ile**/Thr | **ATA**/ACA | n.a. | PR | GU479992 |
| 78 | 235 : **T**/C | 78 : **Leu**/Pro | **CTC**/CCC | n.a. | PR | GU479993 |
| 83 | 251 : **T**/C | 83 : **Pró**/Pro | **CCT**/CCC | n.a. | Roberts *et al.*, 2005 | GU479994[a] |
| 87 | 261 : **G**/A | 87 : **Val**/Ile | **GTA**/ATA | n.a. | Riebold *et al.*, 2004 | AJ746341 |
| 100 | 300 : **G**/T | 100 : **Ala**/Ser | GCA/TCA | n.a. | Riebold *et al.*, 2004 | AJ746341 |
| 111 | 334 : **T**/C | 111: **Ile**/Thr | **ATA**/ACA | No | Lane *et al.* 1997 | PCU66280 |
| 171 | 515 : **A**/G | 171 : **Ser**/Ser | **TCA**/TCG | No | Ma *et al.*, 1999 | AJ586567[b] and AY628435[c] |
| 248 | 745 : **T**/G | 248 : **Val**/Gly | **GTT**/GGT | Yes | Lane *et al.*, 1997 | PCU66279 |

Wild-type nucleotides, amino acids and codons are in bold; nucleotide changes are underlined. ID, sequence variation identification; n.a., not available; PR, present report. GenBank accession no. PCU66282 was considered as the DHPS wild-type sequence (Lane *et al.*, 1997). [a,b,c] GenBank accession numbers correspond to the references where the sequence variations were described for the first time, except for accession numbers GU479994 (present report), AJ586567 (Riebold & Reisinger, 2003) and AY628435 (Iliades *et al.*, 2004).

Table I. – Sequence variations of *P. jirovecii* DHPS gene.

residues Thr[55], Pro[57], Ile[75] and Ile[111] are completely conserved or conservatively related among *Pneumocystis* organisms and other microorganisms, such as *P. falciparum, Bacillus subtilis, Escherichia coli* and *Streptococcus pneumoniae* (Lane *et al.*, 1997; Ma *et al.*, 1999). The DHPS sequence variation detected at codon 23 correlates with a point mutation that confers resistance to sulpha drugs in two bacterial species. DHPS mutations identified at codons 55, 57 and 60 occurred at a "hot spot" region for drug resistance in other organisms (Lane *et al.*, 1997; Ma *et al.*, 1999). A potential association of point mutations at *P. jirovecii* DHPS codons 55 and 57 with sulpha or sulphone resistance has already been reported by several authors. These sequence variations are located at one of the active sites of the enzyme and are similar to point mutations that lead to sulpha drugs resistance in other organisms (Lopez *et al.*, 1987; Brooks *et al.*, 1994; Kazanjian *et al.*, 1998; Triglia *et al.*, 1998; Vedantam *et al.*, 1998; Helweg-Larsen *et al.*, 1999; Armstrong *et al.*, 2000; Huang *et al.*, 2004). For this reason, mutations at codons 55 and 57 are the most studied sequence variations in the *P. jirovecii* DHPS locus. These are no synonymous polymorphisms, which result in amino acid substitutions at positions 55 and 57 (single mutations), or both (combined double mutation). Different strains with single or double amino acid substitutions at these positions have been identified. Several studies demonstrated a statistically significant association between the presence of these point mutations and prior sulpha or sulphone prophylaxis, supporting the hypothesis that DHPS mutations at these sites could reduce sulpha or sulphone sensitivity and lead to sulpha resistance by *P. jirovecii* (Kazanjian *et al.*, 1998; Ma *et al.*, 1999; Armstrong *et al.*, 2000; Kazanjian *et al.*, 2000; Huang *et al.*, 2004). Residues 55 and 57 are located in a highly conserved region, identical among several microorganisms, thought to be involved in binding to the substrate (DHPPP) and to the sulphonamides molecules. Mutations at or very near these positions may be implicated in the re-arrangement of protein structure affecting substrate and sulpha biding to DHPS enzyme. Identical mutations have been shown to confer resistance to sulpha drugs in other microorganisms such as *P. falcipasrum, E. coli*, and *S. pneumoniae* (Lopez *et al.*, 1987; Brooks *et al.*, 1994; Triglia *et al.*, 1998; Vedantam *et al.*, 1998; Ma *et al.*, 1999; Armstrong *et al.*, 2000). The potential effect of sequence variations, other than the mutations at codons 55 and 57, should be investigated in further studies. Mutations suspected to be involved in sulpha or sulphone resistance (ex. point mutations at positions 23, 60 and 111) should be analyzed in further studies in order to clarify their clinical relevance (Lane *et al.*, 1997; Riebold *et al.*, 2006). Also, the clinical importance of synonymous polymorphisms should be explored. It is established that silent polymorphisms could cause interference with protein activity as a result of allele-specific differences in mRNA folding that could influence the splicing process, or the transcriptional control and regulation, or as a consequence of association with a haplotype involving other functional no synonymous polymorphisms (Shen *et al.*, 1999; Kimchi-Sarfaty *et al.*, 2007). Thus, the clinical significance of the DHPS gene variations must be inferred from correlating the clinical outcome with the presence of DHPS gene polymorphisms in patients with PcP.

## ASSOCIATION OF SULPHA OR SULPHONE AGENTS WITH *P. JIROVICII* DHPS GENE MUTATIONS

Failure of sulpha or sulphone prophylaxis against PcP has been reported in up to one fourth of patients (Bonora *et al.*, 1998; Moorman *et al.*, 1998). To determine the role of drug resistance in these failures, investigators, in several studies, examined whether DHPS mutations are more frequent among patients with or without prior exposure to sulpha agents, and whether infections in patients with or without DHPS mutations are more likely to be unresponsive to a sulpha drug (Table II). In total, these studies span a period between 1976 and 2007, involving patients and respective clinical data from United States of America (USA) and several countries in Europe. Eleven of those studies have demonstrated a significant association between the use of sulpha or sulphone agents for PcP prophylaxis in HIV-infected patients and the presence of DHPS mutations (Kazanjian *et al.*, 1998; Ma *et al.*, 1999; Helweg-Larsen *et al.*, 1999; Santos *et al.*, 1999; Kazanjian *et al.*, 2000; Huang *et al.*, 2000; Visconti *et al.*, 2001; Ma *et al.*, 2002; Miller *et al.*, 2003; Nahimana *et al.*, 2003; Zingale *et al.*, 2003) (Table II). However, these studies used different PcP prophylaxis and exposure to sulpha drugs definitions, which limits the possibility of comparing results and detailing the analysis. The first study performed with this purpose was conducted between 1976 and 1997, in USA. The authors reported that DHPS mutations were more common in AIDS patients who received sulpha or sulphone prophylaxis (5/7 – 71 %), compared with those that did not receive sulpha or sulphone prophylaxis (2/13 – 15 %), suggesting that mutations in the *P. jirovecii* DHPS gene were associated with sulpha prophylaxis failure in AIDS patients ($P$ = 0.022); and this difference became even more significant when non-HIV immunocompromised patients (7) and AIDS patients (20) were compared. Mutations were present in two (10 %) out of 20 patients who had not receive prophylaxis and five (71 %) out of seven patients who received a sulpha or sulphone -containing agent ($P$ = 0.0032) (Kazanjian *et al.*, 1998).

In a study that ran from 1985 to 1998, also in USA, a significant association was found between prior exposure to TMP-SMZ or dapsone (in prophylactic dose) and the presence of DHPS mutations at codon 55 and/or codon 57. Mutations were found in 69 % (11/16) of the patients with documented prior exposure, compared with 20 % (3/15) of the patients without such exposure ($P = 0.011$) (Ma *et al.*, 1999). In a third study performed between 1991 and 1997, in USA, AIDS patients who had significant exposure to TMP-SMZ or dapsone prophylaxis were more likely to yield mutant *P. carinii* DHPS sequences ($P = 0.001$). Only 23 % (14/60) of the AIDS patients who did not have significant exposure to prophylaxis with a sulpha or sulphone agent had mutant DHPS genes. In contrast, 76 % (28/37) of the AIDS patients who had significant exposure to sulpha or sulphone prophylaxis demonstrated mutant DHPS genes (Kazanjian *et al.*, 2000). In a prospective study conducted, in the period 1996-1999, in three geographically distinct cities of USA, *P. jirovecii* DHPS mutations were significantly more common ($P < 0.001$) in patients who had previous exposure to sulpha or sulphone prophylaxis (57/71 – 80.3 %) than in those who denied sulpha or sulphone prophylaxis use (19/40 – 47.5 %) (Huang *et al.*, 2000). In a prospective cohort of HIV-1-seropositive patients who had PcP, conducted in 1998-1999, in Denmark, DHPS mutations were significantly more common in patients who had previous exposure to sulpha drugs (18/29 – 62 %) than in those who had no exposure (13/123 – 10.5 %) ($P < 0.0001$) (Helweg-Larsen *et al.*, 1999). In Italy, in a study, conducted between 1992 and 1997, the results obtained suggested that DHPS mutations were significantly associated with failure of anti-*Pneumocystis* sulphone prophylaxis ($P = 0.031$). The authors observed that an increased number of mutant *P. jirovecii* strains were isolated from patients no longer having prophylaxis (Visconti *et al.*, 2001). Another study of *P. jirovecii* isolates, also from Italy, performed in 1994-2001, confirmed the association between DHPS mutations and prior sulpha prophylaxis. Mutations were observed in 19 % (6/31) of patients exposed to sulpha prophylaxis, compared with 4 % (3/76) of patients not exposed to sulpha prophylaxis ($P = 0.017$) (Ma *et al.*, 2002). Also in a third study in Italy, running from 1996 until 2002, mutations were identified in 40 % (28/70) of specimens analyzed and were significantly more common in patients exposed to sulpha drugs (21/29 – 72.4 %) than in those not exposed to sulpha drugs (4/35 – 11.4 %) (Zingale *et al.*, 2003). In a study conducted in the United Kingdom (UK), involving patients studied in different periods, 1992-1993 and 2000-2001, the presence of DHPS mutations (type 2 – mutation at codon 55, type 3 – mutation at codon 57, and type 4 – mutation at codons 55 plus 57) was associated with exposure to a sulpha drug during the three months before the episode of PCP ($P = 0.01$). Furthermore, for all patients, genotype 4 was associated with sulpha drug exposure ($P < 0.0005$). Among isolates from patients diagnosed in 1992-1993, exposure to dapsone prophylaxis was particularly associated with DHPS genotype 4 ($P < 0.002$). A quarter of all isolates collected in 1992-1993, from patients not exposed to sulpha, were of mutant genotypes (types 2, 3, and 4), suggesting selection pressure. There was reversal of the mutant-to-wild type genotype ratios when selection pressure was removed, as in 2000-2001. Based on this data the authors deduced that human *Pneumocystis* infection arises by recent transmission, because if reactivation of latent infection was the explanation, then no differences in DHPS genotypes would be observed over time or by geographical location, irrespective of patients' receipt of sulpha drugs (Miller *et al.*, 2003). In a study conducted in France, involving patients' pulmonary specimens obtained between 1993 and 1998, the authors found a statistically significant association ($P < 0.01$) between DHPS gene mutations and prophylaxis with TMP-SMZ or dapsone (Santos *et al.*, 1999). Also in France, in a study running from 1993 until 1996, all patients who developed PcP while receiving pyrimethamine/sulphadoxine (PM/SD) prophylaxis (n = 14) had a strain harbouring DHPS with a mutation at codon 57. This mutation was only present in 14 % (20/144) of patients not receiving prophylaxis ($P < 0.001$) (Nahimana *et al.*, 2003).

It should be noted that in all studies analyzed until now, in this review, DHPS mutations were detected in PcP patients who were not currently receiving sulpha or sulphone agents, including those newly diagnosed with HIV infection, aside from the patients currently exposed to sulpha or sulphone drugs. However those patients were only a minority. Also, there are studies where association between previous prophylaxis with sulpha drugs and the presence of mutations in the DHPS gene could not be found. In a study performed in France, in the period of 1998-2001, authors could not find correlation between previous prophylaxis with sulpha drugs and the presence of DHPS mutations ($P = 0.55$). Of the isolates with a mutant DHPS type, 75 % (12/16) corresponded to patients who had never received sulpha or sulphone prophylaxis (Latouche *et al.*, 2003). Another study of *P. jirovecii* isolates conducted in Portugal, in 1994-2001, presented similar results. In the studied population, DHPS gene mutations were not significantly more frequent in patients exposed to sulpha drugs compared with patients not exposed ($P = 0.39$) (Costa *et al.*, 2003). And these observations were corroborated in a latter study, also in this country (Esteves *et al.*, 2010). Also in a study conducted in Italy, in 1994-2004, no association was reported between previous sulpha prophylaxis and the degree of *P. jirovecii* DHPS mutations, 71.4 % of isolates with a mutant DHPS genotype corresponded to patients who had never received

| Country | Period of time | DHPS mutations / PcP cases (%) | DHPS mutations / Patients exposed to sulpha drugs (%) | Sulpha / Sulphone agent | DHPS mutations / Patients not exposed to sulpha drugs (%) | P | Reference |
|---|---|---|---|---|---|---|---|
| **North America** | | | | | | | |
| USA | 1976-1997 | 7/27 (26 %)†§ | 5/7 (71 %)* | TMP-SMZ, Dapsone | 2/20 (10 %) | 0.0032 | Kazanjian *et al.*, 1998 |
| USA | 1985-1998 | 16/37 (43 %)† | 11/16 (69 %)** | TMP-SMZ, Dapsone | 3/15 (20 %) | 0.011 | Ma *et al.*, 1999 |
| USA | 1995-1998 | 152/220 (69 %)§ | n.a. | n.a. | n.a. | n.a. | Beard *et al.*, 2000 |
| USA | 1991-1999 | 42/97 (43 %)§ | 28/37 (76 %) | TMP-SMZ, Dapsone | 14/60 (23 %) | < 0.001 | Kazanjian *et al.*, 2000 |
| USA | 1996-1999 | 76/111 (69 %)§ | 57/71 (80 %) | TMP-SMZ, Dapsone | 19/40 (48 %) | < 0.001 | Huang *et al.*, 2000 |
| USA | 1983-2001 | 58/145 (40 %) | n.a. | TMP-SMZ, Dapsone | n.a. | n.a. | Kazanjian *et al.*, 2004 |
| USA | 1997-2002 | 175/215 (81 %) | n.a. | n.a. | n.a. | n.a. | Crothers *et al.*, 2005 |
| **Europe** | | | | | | | |
| Denmark | 1989-1999 | 31/152 (20 %) | 18/29 (62 %) | TMP-SMZ | 13/123 (11 %) | < 0.0001 | Helweg-Larsen *et al.*, 1999 |
| UK | 1992-1993 | 9/25 (36 %) | 5/9 (55.6 %) | TMP-SMZ, Dapsone | 4/16 (25 %) | < 0.01 | Miller *et al.*, 2003 |
| UK | 2000-2001 | 2/12 (17 %) | 0/0 | TMP-SMZ, Dapsone | 2/12 (17 %) | n.a. | Miller *et al.*, 2003 |
| Italy | 1992-1997 | 7/20 (35 %) | 4/5 (80 %) | Dapsone+ pyrimethamine | 3/15 (20 %) | 0.031 | Visconti *et al.*, 2001 |
| Italy | 1994-2001 | 9/107 (8.4 %) | 6/31 (19.4 %) | TMP-SMZ | 3/76 (4 %) | 0.017 | Ma *et al.*, 2002 |
| Italy | 1996-2002 | 28/70 (40 %) | 21/29 (72.4 %) | TMP-SMZ, Dapsone | 4/35 (11.4 %) | < 0.0001 | Zingale *et al.*, 2003 |
| Italy | 1994-2004 | 14/154 (9.1 %) | 4/38 (10.5 %) | TMP-SMZ | 10/116 (8.6 %) | 0.74 | Valerio *et al.*, 2007 |
| France | 1993-1998 | 17/30 (57 %) | 5/5 (100 %) | TMP-SMZ, Dapsone | 3/12 (25 %) | < 0.01 | Santos *et al.*, 1999 |
| France | 1993-1996 | 57/158 (36.1 %)† | 16/20 (80 %) | Tmp-smz | 41/138 (30 %) | < 0.001 | Nahimana *et al.*, 2003 |
| France | 1998-2001 | 16/92 (17.4 %) | 2/15 (13.3 %) | TMP-SMZ | 12/73 (16.4 %) | 0.55 | Latouche *et al.*, 2003 |
| Portugal | 1994-2001 | 24/89 (27 %) | 6/17 (35.5 %) | TMP-SMZ | 18/72 (25 %) | 0.39 | Costa *et al.*, 2003 |
| Portugal | 2001-2004 | 4/55 (7 %)† | n.a. | n.a. | n.a. | n.a. | Esteves *et al.*, 2008 |

Table II – Prevalence of *Pneumocystis jirovecii* DHPS mutations in immunocompromised patients.

Table II continued

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Europe** | | | | | | | |
| Portugal | 2001-2007 | 6/67(9 %) | n.a. | TMP-SMZ | n.a. | > 0.05 | Esteves *et al.*, 2010 |
| Spain | 2001-2003 | 5/15 (33.3 %) | n.a. | No exposure | n.a. | n.a. | Montes-Cano *et al.*, 2004 |
| Spain | 2001-2004 | 8/36 (22 %)† | n.a. | n.a. | n.a. | n.a. | Esteves *et al.*, 2008 |
| Spain | 2000-2004 | 7/188 (3.7 %)§ | 6/184 (3.3 %) | TMP-SMZ | 1/4 (25 %) | n.a. | Alvarez-Martinez *et al.*, 2008 |
| **Asia** | | | | | | | |
| Japan | 1994-1999 | 6/24 (25 %)† | n.a. | Pentamidine | n.a. | n.a. | Takahashi *et al.*, 2000 |
| China | 1998-2001 | 1/15 (7 %) | n.a. | No exposure | n.a. | n.a. | Kazanjian *et al.*, 2004 |
| China | 2007-2008 | 0/10 | 0/0 | No exposure | 0/10 | n.a. | Li *et al.*, 2009 |
| India | Jun-Nov 2006 | 0/4 | 0/0 | No exposure | 0/4 | n.a. | Tyagi *et al.*, 2008 |
| Thailand | 1997-2003 | 2/17 (11.7 %) | n.a. | n.a. | n.a. | n.a. | Siripattanapipong *et al.*, 2008 |
| **Africa** | | | | | | | |
| Zimbabwe | 1992-1993 | 1/14 (7.1 %) | 0/0 | No exposure | 1/14 (7.1 %) | n.a. | Miller *et al.*, 2003 |
| South Africa | 2000-2002 | 4/30 (13.3 %), children | 0/1 (0 %) | n.a. | 4/29 (13.8 %) | n.a. | Zar *et al.*, 2004 |
| South Africa | 2000-2003 | 2/53 (3.8 %) | n.a. | TMP-SMZ | n.a. | n.a. | Robberts *et al.*, 2005 |
| South Africa | 2006-2007 | 81/151 (56 %) | n.a. | n.a. | n.a. | n.a. | Dini *et al.*, 2010 |
| **South America** | | | | | | | |
| Brazil | 1997-2004 | 0/57 | 0/5 | n.a. | 0/52 | n.a. | Wissman *et al.*, 2006 |
| **Oceania** | | | | | | | |
| Australia | 2001-2007 | 8/60 (13.3 %)† | 2/8 (25 %) | TMP-SMZ | 6/52 (12 %) | n.a. | van Hal *et al.*, 2009 |

n.a. Not available; PM/SD – Pyrimethamine/Sulphadoxine.
* Two patients with mutations were successfully treated with sulpha/sulphone agent in therapeutic dose.
** No correlation was found between survival after initiation of therapy with a sulphonamide or dapsone containing regimen and the presence of mutations at codon 55 and/or 57.
† HIV-infected patients and non-HIV-infected patients.
§ Multicenter study.

previous sulpha prophylaxis and who developed PcP from 2000 to 2004 (Valerio *et al.*, 2007). In a study performed in 2004, DHPS gene mutations were detected in three different groups (archival specimens from 13 immunodeficient adults with PcP, eight adults colonized by *P. jirovecii* and 19 immunocompetent infants, with bronchiolitis and infected with *P. jirovecii*) of patients, although none of them had prior exposure to sulphonamide drugs. Because of the young age of the infants and consequently their short medical history, it was easy to ascertain that none had been subjected to prior sulphonamide exposure. On the other hand, the exposure in adults throughout their lifetime cannot strictly be ruled out. Nevertheless, in this study, no adults were treated with sulphonamides in the three months preceding specimen retrieval (Totet *et al.*, 2004).

All these observations strongly suggest that the mutations are selected by drug pressure, and that their acquisition and transmission can be incidental, either by person-to-person transmission or from an environmental source. The Totet and collaborators study is an example of this theory, reporting DHPS mutations in patients, which included an infant group, with no prior exposure to sulpha or sulphone agents, (Totet *et al.*, 2004). The fact that, in a study, the city of patient residence has been identified as an independent risk factor for having a mutant *P. jirovecii* DHPS gene mutation, also supports this hypothesis (Huang *et al.*, 2000). Moreover, hospitalization in a specific hospital was an independent risk factor for having *P. jirovecii* harbouring the same DHPS mutation, which indirectly supports that interhuman transmission, may affect the dissemination of the mutant strains (Nahimana *et al.*, 2003). Therefore, analysis of the DHPS locus can also be used as a marker for studying the potential circulation of *P. jirovecii* within the human reservoir.

## PREVALENCE OF *P. JIROVECII* DHPS MUTATIONS

The DHPS locus has been studied extensively in recent years because of the occurrence of mutations that may be a consequence of exposure to sulpha or sulphone agents, and that are related to the emergence of resistant strains of *P. jirovecii* to these drugs. The *P. jirovecii* DHPS gene has been amplified and characterized by using PCR followed by DNA sequencing, restriction fragment length polymorphism (RFLP) or single strand conformation polymorphism (SSCP) (Beard *et al.*, 2000; Hauser *et al.*, 2001; Costa *et al.*, 2003). These techniques have been applied in several studies with the purpose to determine mutations rates.

In industrialized countries like USA, European countries, Japan and Australia, studies involving HIV-infected patients with PcP reported rates between 3.7 % and 81 % (Table II). Higher *P. jirovecii* DHPS gene mutation rates (between 26 % and 81 % [mean 53 %]) were reported in HIV-infected patients with PcP in USA (Kazanjian *et al.*, 1998; Ma *et al.*, 1999; Beard *et al.*, 2000; Kazanjian *et al.*, 2000; Huang *et al.*, 2000; Kazanjian *et al.*, 2004; Crothers *et al.*, 2005). Seven studies were conducted in different cities of the country, spanning a period between 1976 and 2002. The lowest rate of DHPS mutations (26 %) was reported in a study that involved patients from Indiana and Michigan studied between 1976 and 1997 (Kazanjian *et al.*, 1998). DHPS mutations were detected in 43 % of HIV-infected and non-HIV-infected immunodeficient patients studied in the period of 1985-1998 (Ma *et al.*, 1999). In a multicenter study, performed by the same authors, involving four cities Detroit, Indiana, Boston and Denver, in the period of 1991-1999, 43 % was the overall percentage of patients with DHPS mutations reported. However this percentage varied according to geographic locale, 75 % in Detroit, 24 % in Indiana, 54 % in Boston and 36 % in Denver (Kazanjian *et al.*, 2000). Also in 2000 it was published another multicenter study involving five other cities Atlanta, Cincinnati, Los Angeles, San Francisco and Seattle. DHPS mutations were detected with an overall prevalence of 69 % (Beard *et al.*, 2000). Another multicenter study, involving patients from Atlanta, Seattle and San Francisco, spanning the period 1996-1999, found an overall prevalence of DHPS mutations of 69 %. When stratified by study site the rates of *P. jirovecii* DHPS mutations were of 54.2 % in Atlanta, 77.8 % in Seattle and 81.5 % in San Francisco (Huang *et al.*, 2000). One more multicenter study, covering the period 1983-2001, analysed specimens from patients from Michigan, Boston, Indianapolis, Detroit and Denver. *P. jirovecii* DHPS mutations were detected in 40 % of the PcP patients. Mutation prevalence increased to 70 % during 2000-2001, from 25 % during 1994-1995 ($P < 0.01$) (Kazanjian *et al.*, 2004). The highest rate of DHPS mutation (81 %) was reported in San Francisco, in the time period 1997-2002 (Crothers *et al.*, 2005).

In Europe, fourteen studies covering a period between 1989 and 2007, reported *P. jirovecii* DHPS gene mutation rates of 3.7-57 % (mean 23 %) (Table II). In Denmark, it was reported a prevalence of *P. jirovecii* DHPS mutations of 20 %, in HIV-infected patients with PcP followed between 1989 and 1999 (Helweg-Larsen *et al.*, 1999). In a study conducted in the UK, a decline in the proportion of *P. jirovecii* DHPS mutations was observed for the two time periods 1992-1993 (36 %) and 2000-2001 (17 %) of the study. The explanation for this decline was attributed to the decreased use of TMP-SMZ in the general population, since July 1995 in this country (Miller *et al.*, 2003). In Italy, HIV-infected patients with PcP from two cities Rome and Milan, presented rates of DHPS mutations of 35 % in Rome and 8.4 to 40 %,

in three studies involving patients followed in two different hospitals in Milan, between 1992 and 2004. These differences of percentages were justified by the difference in sensitivity of the protocols used for targeting of the DHPS gene (Zingale *et al.*, 2003); and by the lower use of dapsone in prophylaxis among patients in northern Italy, or by differences in the adherence level to prophylaxis, unknown differences related to the geographic origin of patients, or epidemiology of PcP transmission (Valerio *et al.*, 2007). Also in France, a decrease of the DHPS mutation rates was found. In two studies performed in different cities, spanning similar periods of time, 1993-1998 and 1993-1996, the percentages reported were high, 57 and 36.1 %, respectively; but in the third study, covering the period 1998-2001, a lower percentage was reported 17.4 % (Santos *et al.*, 1999; Nahimana *et al.*, 2003; Latouche *et al.*, 2003). The great majority of the population (HIV-infected and non-HIV-infected patients) examined, in the period 1998-2001, had never received supha or sulphone prophylaxis, on the contrary of the patients of the retrospective study performed by Santos and collaborators (Latouche *et al.*, 2003). A decline in the proportion of isolates from patients (HIV-infected and non-HIV-infected) with DHPS mutations was observed in three studies developed in Portugal, in the period 1994-2007, from 27 %, in the period 1994-2001, to 7-9 %, in 2001-2007. This reduction was attributed to the decreased use of sulpha prophylaxis after the introduction of HAART (Costa *et al.*, 2003; Esteves *et al.*, 2008, 2010). In Spain, also three studies were reported, involving HIV-infected and non-HIV-infected patients, spanning the period 2000-2004. The higher rates (33.3 and 22 %) of mutations were found in two studies in Seville, and the lower rate (3.7 %) was reported in a prospective multicenter study involving 12 Spanish hospitals in different cities. The explanation for this decline was attributed to the fact that in nearly 50 % of the patients enrolled in the multicenter study HIV infection manifested itself with an episode of PcP (Montes-Cano *et al.*, 2004; Esteves *et al.*, 2008; Alvarez-Martinez *et al.*, 2008).

In Japan, in a study performed between 1994 and 1999, 25 % of the HIV-infected patients and non-HIV-infected immunodeficient patients studied with PcP presented *P. jirovecii* DHPS gene mutations (Table II) (Takahashi *et al.*, 2000). Also in Australia, a lower percentage of *P. jirovecii* DHPS mutations (13.3 %) was reported. The study, that took place between 2001 and 2007, involved HIV-infected patients and non-HIV-infected immunodeficient patients (Table II).

European specimens showed a higher prevalence of the wild-type sequence and a lower frequency of mutations than in the USA. These data suggest that there could be geographical variation in the prevalence of *P. jirovecii* DHPS gene mutations, possibly caused by intrinsic epidemiological factors that influence the circulation and transmission of different genotypes, or perhaps because of differing use of sulpha or sulphone drugs for PcP prophylaxis (Kazanjian *et al.*, 1998; Armstrong *et al.*, 2000; Beard *et al.*, 2000; Miller *et al.*, 2003; Costa *et al.*, 2003; Esteves *et al.*, 2008). Also in European countries a decline in the DHPS gene mutation frequency has already been described in the UK, France and Portugal in two different time periods (Miller *et al.*, 2003; Latouche *et al.*, 2003; Costa *et al.*, 2003; Esteves *et al.*, 2008), which may be reflecting the decreased use of prophylaxis with sulpha or sulphone drugs, following the introduction and widespread use of HAART in these countries (Morris *et al.*, 2004).

In developing countries, lower rates of *P. jirovecii* DHPS mutations were reported (Table II). In China two studies were performed in different cities with about 10 years of interval. In the first, enrolled between 1998 and 2001, 7 % was the rate of DHPS mutations detected in AIDS patients from Beijing (Kazanjian *et al.*, 2004). The second study was conducted in Guangzhou, between 2007 and 2008, and no DHPS mutations were observed (Li *et al.*, 2009). These results suggested that the prevalence of DHPS mutations in China may be low due to limited use of sulpha drugs (Li *et al.*, 2009). Also in India no *P. jirovecii* DHPS mutations were detected, in a study conducted for six months in 2006, including HIV-infected patients and non-HIV-infected patients with PcP (Tyagi *et al.*, 2008). Nevertheless, in Thailand, 11.7 % of the HIV-infected patients studied, in the period 1997-2003, presented DHPS mutations (Siripattanapipong *et al.*, 2008). In the African continent, three studies have been developed with the purpose to determine the *P. jirovecii* DHPS mutations rates in patients with PcP. One study conducted in Zimbabwe, for one year (1992-1993), described a prevalence of 7.1 % of DHPS mutations in HIV-infected patients with PcP (Miller *et al.*, 2003). In South Africa two studies ran in similar periods, 2000-2002 and 2000-2003, enrolling different populations (HIV-infected children and adults with PcP), in different regions of the country, with the purpose to determine the prevalence of *P. jirovecii* DHPS mutations. In children the rate was 13.3 %, while in the adults it was 3.8 %. These low prevalence were attributed to the lack of exposure to TMP-SMZ in the populations studied, especially the children (Zar *et al.*, 2004; Robberts *et al.*, 2005). However, in a recent study performed between 2006 and 2007 involving a large group (712) of HIV-infected adults suspected of PcP from a different province (Gauteng Province), pneumocystosis was diagnosed in 24 % of the patients, and 56 % of which presented with *P. jirovecii* DHPS mutations (Dini *et al.*, 2010). These authors attributed the higher percentage of *P. jirovecii* DHPS mutations, in comparison to the two previous studies performed in the same country, to the higher rates of HIV prevalence in this province, and to the increased widespread empirical use of TMP-SMZ in South Africa.

In Brazil, no *P. jirovecii* DHPS mutations were detected, in a study conducted in 1997-2004, including AIDS patients with PcP. These results were also attributed to the low level of exposure to sulpha prophylaxis in that country (Wissman *et al.*, 2006).

The similar low rates of *P. jirovecii* DHPS mutations reported in developing countries suggest that selective pressure on local strains may not have reached the levels found in USA and Europe. South Africa seems to be an exception, at least in some provinces, where high rates of *P. jirovecii* DHPS mutations were found. Since the implementation of PcP prophylaxis in these settings is still limited, this discrepancy, in relation to the other developing countries where studies were performed, seems to be associated with the extensive use of sulpha agents, especially TMP-SMZ, against diseases other than PcP. The high prevalence of HIV infection in South Africa could also contribute to the acceleration of the interhuman transmission of *P. jirovecii* and the concomitant circulation of DHPS mutations in this susceptible population (Dini *et al.*, 2010). Thus, the differing use of sulpha or sulphone agents for PcP prophylaxis, and/or for treatment of other diseases, could be an important factor that contributes to the varying rates of mutation reported in different geographical regions.

## CLINICAL IMPORTANCE OF DHPS GENE MUTATIONS IN *P. JIROVECII*

Several studies have examined the effect of DHPS gene mutations on PcP clinical outcomes such as death, death specifically attributable to PcP, and PcP treatment failure with sulpha or sulphone agents, presenting inconsistent results. Whether the presence of DHPS gene mutations confers clinical resistance to sulpha or sulphone drugs for PcP treatment remains unclear. The majority of the studies conducted in this area reported the association between DHPS mutations and prior sulpha or sulphone prophylaxis. But also, some of the results obtained until now suggest that higher concentrations (therapeutic dose) of sulpha agents in pulmonary tissue can result in successful treatment of most patients with PcP who harbour DHPS gene mutations (Kazanjian *et al.*, 1998; Mei *et al.*, 1998; Helweg-Larsen *et al.*, 1999; Ma *et al.*, 1999; Kazanjian *et al.*, 2000; Navin *et al.*, 2001; Visconti *et al.*, 2001; Ma *et al.*, 2002; Latouche *et al.*, 2003; Meshnick & Kazanjian, 2005; Valerio *et al.*, 2007; Alvarez-Martinez *et al.*, 2008). A few studies reported significantly more therapy failure, when administering sulpha or sulphone agents, in patients with mutant *P. jirovecii* than in patients with wild-type (Helweg-Larsen *et al.*, 1999; Kazanjian *et al.*, 2000; Valerio *et al.*, 2007). Valerio and collaborators study, suggested that mutations in the *P. jirovecii* DHPS gene were associated with possible failure of anti-*P. jirovecii* high-dose sulpha therapy. Patients unsuccessfully treated with sulpha harboured a significantly higher ($P = 0.04$) number of DHPS mutations than those successfully treated. Even so, these authors observed that 46.2 % of DHPS-mutated PcP events were successfully treated, being reasonable to assume that the high drug concentration achieved in lung tissues by SMZ (possibly exceeding the therapeutic dose for wild type) guaranteed the success of treatment in those patients (Valerio *et al.*, 2007).

Concerning PcP clinical outcomes, in a multivariate analysis, a higher death rate (3-months survival rate) was reported in patients who had mutant *P. jirovecii* compared to patients with the wild-type DHPS, after important mortality cofactors like age, $CD4^+$ T cells count, and arterial oxygen partial pressure ($PaO_2$) were controlled for (Helweg-Larsen *et al.*, 1999). Also van Hal and collaborators reported associations between *P. jirovecii* DHPS mutations and poor outcomes (death) compared to patients with wild-type DHPS (van Hal *et al.*, 2009). But this correlation could not be confirmed in other studies. Visconti and collaborators could not find a statistically significant difference in severity or outcome of the pneumonia caused by wild-type or mutant DHPS (Visconti *et al.*, 2001). Also Navin and collaborators found no association between the presence of DHPS mutations and overall number of deaths at six weeks and death attributable specifically to PcP (Navin *et al.*, 2001). Ma and collaborators could not find a significant association between the presence of DHPS mutations and mortality (Ma *et al.*, 2002). In a study, with univariate analysis, the rates of death attributed to PcP was similar in patients who had a *P. jirovecii* mutant DHPS and those who did not (Nahimana *et al.*, 2003). In another study, there was no significant difference in favourable (after 14 days of treatment; long-term favourable – after more than 14 days of treatment with or without change of therapeutic agent) or adverse outcome (death) in PcP caused by wild or mutant DHPS genotypes (Latouche *et al.*, 2003). Alvarez-Martine and collaborators could not find, also, an association between *P. jirovecii* DHPS mutations and worse outcome. Treatment with TMP-SMZ was successful in all of their patients who harboured DHPS mutations and who were treated with this drug, and were still alive six months later (Alvarez-Martinez *et al.*, 2008).

Some authors described selected clinical variables with a possible relation to the severity of PcP. The outcome and mortality of AIDS patients with PcP harbouring DHPS gene mutations were related primarily to the underlying severity of illness and the initial severity of PcP, more than to the presence of mutations (Crothers *et al.*, 2005). Comparing these results with a prior study conducted by the same group, where patients with DHPS gene mutations were less likely to die and to fail

TMP-SMZ therapy (Navin *et al.*, 2001), these authors were unable to explain this apparent qualitative reversal, since their clinical practice was the same and the DHPS mutations observed were unchanged. At presentation, a low serum albumin (< 30 g/l – $P$ = 0.004) and the requirement for early intensive care unit (ICU) admission ($P$ = 0.012) were, by multivariate analysis, independent predictors of mortality (Crothers *et al.*, 2005). These results are consistent with latter studies (Valerio *et al.*, 2007; Alvarez-Martinez *et al.*, 2008). A higher lactate dehydrogenase (LDH) and the need for intubation at admission were considered, also, as predictors of mortality in these patients (Valerio *et al.*, 2007; Alvarez-Martinez *et al.*, 2008; van Hal *et al.*, 2009). And $PaO_2$ < 60 mm Hg at admission ($P$ = 0.02) and requirement of adjunctive corticosteroids ($P$ = 0.03) were also factors influencing outcome and mortality of patients who died of PcP (Alvarez-Martinez *et al.*, 2008).

Some studies raised questions about choice or dose of sulpha agents, and duration of exposure to the drug. Kazanjian and collaborators described no significant associations between choice and dose of sulpha agent (i.e., dapsone vs TMP/SMX), but in contrast, they reported that the increased duration of sulpha or sulphone as prophylaxis raised the chance of having a DHPS mutation (Kazanjian *et al.*, 2000). No associations between the presence of DHPS mutations and demographic factors (sex, age, and race) were detected (Kazanjian *et al.*, 2000; Ma *et al.*, 2002; Esteves *et al.*, 2010; Dini *et al.*, 2010). Also there were no associations between $CD4^+$ T cells count, HIV viral loads, and prior episodes of PCP and *P. jirovecii* DHPS mutations (Kazanjian *et al.*, 2000; Huang *et al.*, 2000; Ma *et al.*, 2002; Esteves *et al.*, 2010). After all these studies, questions like: whether the presence of DHPS single or double gene mutations confers clinical resistance to sulpha or sulphone agents on higher therapeutic doses for PcP treatment; whether the presence of DHPS single or double mutations have differential effects on PcP clinical outcome; whether drug-susceptibility dependent on polymorphism(s) that occur in one region of the genome or in various regions of the genome of the organism, remain to be answered

## CONCLUSION

Several studies confirmed a significant association between the presence of point mutations at *P. jirovecii* DHPS codons 55 and 57 and prior sulpha or sulphone prophylaxis, supporting the hypothesis that DHPS mutations at these sites could reduce sulpha or sulphone sensitivity and lead to sulpha resistance by *P. jirovecii*. However, therapeutic dose of sulpha agents can result in successful treatment of most patients with PcP who harbour DHPS gene mutations, raising the hypothesis of the existence of another mutation in the DHPS gene or in a related gene that might be necessary to confer clinically significant resistance. The presence of *P. jirovecii* mutant genotypes in persons who have not been exposed to sulpha or sulphone agents could also have important implications for the transmission and epidemiology of this infection.

The outcome of PcP is a complex issue, with multiple factors affecting the patients' prognosis, varying from full recovery to death. The severity of *P. jirovecii* pneumonia might be affected by hypoxemia, higher LDH, low serum albumin, the requirement for early ICU admission and the need for intubation also at admission, and by underlying or concurrent illnesses.

Although we are assisting to a decline in the incidence of PcP and consequently to the *P. jirovecii* DHPS mutations rates in the developed countries, due to the earlier detection of HIV, and the widespread use of PcP prophylaxis and HAART, there is an increased incidence of PcP in developing regions of the Globe, where most HIV-infected persons reside. In these places HAART and PcP prophylaxis are still being implemented. Therefore the worldwide spread of sulpha drug use could be responsible for an increased incidence of sulpha-resistant microorganisms, including *P. jirovecii*, in the future.

## REFERENCES


Alvarez-Martínez M.J., Moreno A., Miró J.M., Valls M.E., Rivas P.V., De Lazzari E., Sued O., Benito N., Domingo P., Ribera E., Santín M., Sirera G., Segura F., Vidal F., Rodríguez F., Riera M., Cordero M.E., Arribas J.R., Jiménez De Anta M.T., Gatell J.M., Wilson P.E., Meshnick S.R.; Spanish Pcp Working Group. *Pneumocystis jirovecii* pneumonia in Spanish HIV-infected patients in the combined antiretroviral therapy era: prevalence of dihydropteroate synthase mutations and prognostic factors of mortality. *Diagnostic Microbiology and Infectious Diseases*, 2008, *62* (1), 34-43.

Armstrong W., Meshnick S. & Kazanjian P. *Pneumocystis carinii* mutations associated with sulfa and sulfone prophylaxis failures in immunocompromised patients. *Microbes and Infection*, 2000, *2*, 61-67.

Barry S.M. & Johnson M.A. *Pneumocystis carinii* pneumonia: a review of current issues in diagnosis and management. *HIV Medicine*, 2001, *2*, 123-132.

Beard C.B., Carter J.L., Keely S.P., Huang L., Pieniazek N.J., Moura I.N., Roberts J.M., Hightower A.W., Bens M.S., Freeman A.R., Lee S., Stringer J.R., Duchin J.S., Del Rio C., Rimland D., Baughman R.P., Levy D.A., Dietz V.J., Simon P. & Navin T.R. Genetic variation in *Pneumocystis carinii* isolates from different geographic regions: implications for transmission. *Emerging Infectious Diseases*, 2000, *6*, 265-272.

Benson C.A., Kaplan J.E., Masur H., Pau A. & Holmes K.K. Treating opportunistic infections among HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association/ Infectious Diseases Society of America. *MMWR Recommendations and Reports*, 2004, *53*, 1-112.

Bonora S., Di Perri G., Vento S., Cazzadori A. & Concia E. Failure of prophylaxis against PCP in patients with HIV infection. *AIDS Patient Care and STDs*, 1998, *12*, 843-848.

Bozzette S.A., Finkelstein D.M., Spector S.A., Frame P., Powderly W.G., He W., Phillips L., Craven D., Van Der Horst C. & Feinberg J. A randomized trial of three antipneumocystis agents in patients with advanced human immunodeficiency virus infection. *New England Journal of Medicine*, 1995, *332*, 693-699.

Brooks D.R., Wang P., Read M., Watkins W.M., Sims P.F. & Hyde J.E. Sequence variation of the hydroxymethyldihydropterin pyrophosphokinase: dihydropteroate synthase gene in lines of the human malaria parasite, *Plasmodium falciparum*, with differing resistance to sulfadoxine. *European Journal of Biochemistry*, 1994, *224*, 397-405.

Bushby S.R.M. Antibacterial activity, in: Inhibition of Folate Metabolism in Chemotherapy. Hitchings G.H. (ed.), Springer-Verlag, New York, 1983, 75-105.

Centers for Disease Control. Recommendations for prophylaxis against *Pneumocystis carinii* pneumonia for adults and adolescents infected with human immunodeficiency virus. *Morbidity and Mortality Weekly Report*, 1992, *41*, 1-11.

Costa M.C., Helweg-Larsen J., Lundgren B., Antunes F. & Matos O. Mutations in the dihydropteroate synthase gene of *Pneumocystis jirovecii* isolates from Portuguese patients with Pneumocystis pneumonia. *International Journal of Antimicrobial Agents*, 2003, *22* (5), 516-520.

Crothers K., Beard C.B., Turner J., Groner G., Fox M., Morris A., Eiser S. & Huang L. Severity and outcome of HIV-associated *Pneumocystis* pneumonia containing *Pneumocystis jirovecii* dihydropteroate synthase gene mutations. *AIDS*, 2005, *19*, 801-805.

Dini L., du Plessis M., Frean J. & Fernandez V. High prevalence of dihydropteroate synthase mutations in *Pneumocystis jirovecii* isolated from patients with *Pneumocystis* pneumonia in South Africa. *Journal of Clinical Microbiology*, 2010, *48*, 2016-2021.

Esteves F., Gaspar J., Tavares A., Moser I., Antunes F., Mansinho K. & Matos O. Population structure of *Pneumocystis jirovecii* isolated from immunodeficiency virus-positive patients. *Infection Genetics and Evolution*, 2010, *10*, 192-199.

Esteves F., Montes-Cano M.A., De La Horra C., Costa M.C., Calderón E.J., Antunes F. & Matos O. *Pneumocystis jirovecii* multilocus genotyping profiles in patients from Portugal and Spain. *Clinical Microbiology and Infection*, 2008, *14*, 356-362.

French N., Kaleebu P., Pisani E. & Whitworth J.A.G. Human immunodeficiency virus (HIV) in developing countries. *Annals of Tropical Medicine and Parasitology*, 2006, *100*, 433-454.

Hauser P.M., Sudre P., Nahimana A. & Francioli P., and The Study Group. Prophylaxis failure is associated with a specific *Pneumocystis carinii* Genotype. *Clinical Infectious Diseases*, 2001, *33*, 1080-1082.

Helweg-Larsen J., Benfield T.L., Eugen-Olsen J., Lundgren J.D. & Lundgren B. Effects of mutations in *Pneumocystis carinii* dihydropteroate synthase on outcome of AIDS-associated *P. carinii* pneumonia. *Lancet*, 1999, *354*, /1347-1351.

Huang L., Beard C.B., Creasman J., Levy D., Duchin J.S., Lee S., Pieniazek N., Carter J.L., Del Rio C., Rimland D. & Navin T.R. Sulfa or sulfone prophylaxis and geographic region predict mutations in the *Pneumocystis carinii* dihydropteroate synthase gene. *The Journal of Infectious Diseases*, 2000, *182*, 1192-1198.

Huang L., Crothers K., Atzori C., Benfield T., Miller R., Rabodonirina M., Helweg-Larsen J. Dihydropteroate synthase gene mutations in *Pneumocystis* and sulfa resistance. *Emerging Infectious Diseases*, 2004, *10*, 1721-1728.

Iliades P., Walker D.J., Castelli L., Satchell J., Meshnic S.R. & Macreadie I.G. Cloning of the *Pneumocystis jirovecii* trifunctional FAS gene and complementation of its DHPS activity in *Escherichia coli*. *Fungal Genetic and Biology*, 2004, *41*, 1053-1062.

Kaneko Y., Suwa A., Ikeda Y. & Hirakata M. *Pneumocystis jiroveci* pneumonia associated with low-dose methotrexate treatment for rheumatoid arthritis: report of two cases and review of the literature. *Rheumatology*, 2006, *16,* 36-38.

Kaplan J.E., Benson C., Holmes K.H., Brooks J.T., Pau A. & Masur H. Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. Centers for Disease Control and Prevention (CDC); National Institutes of Health; HIV Medicine Association of the Infectious Diseases Society of America. *MMWR Recommendations and Reports*, 2009, *58* (RR-4), 1-207, quiz CE1-4.

Kaplan J.E., Hanson D., Dworkin M.S., Frederick T., Bertolli J., Lindegren M.L., Holmberg S. & Jones J.L. Epidemiology of human immunodeficiency virus-associated opportunistic infections in the United States in the era of highly active antiretroviral therapy. *Clinical Infectious Diseases*, 2000, *30* (Suppl. 1), S5-S14.

Kaplan J.E., Masur H.& Holmes K.K. Guidelines for preventing opportunistic infections among HIV-infected persons-2002. Recommendations of the U.S. Public Health Service and the Infectious Diseases Society of America. *MMWR Recommendations and Reports*, 2002, *51* (RR-8), 1-52.

Kazanjian P., Armstrong W., Hossler P.A., Burman W., Richardson J., Lee C.H., Crane L., Katz J. & Meshnick S.R. *Pneumocystis carinii* mutations are associated with duration of sulfa or sulfone prophylaxis exposure in AIDS patients. *The Journal of Infectious Diseases*, 2000, *182*, 551-557.

Kazanjian P., Locke A.B., Hossler P.A., Lane B.R., Bartlett M.S., Smith J.W., Cannon M. & Meshnick S.R. *Pneumocystis carinii* mutations associated with sulfa and sulfone prophylaxis failures in AIDS patients. *AIDS*, 1998, *12*, 873-878.

Kazanjianp.H., Fisk D., Armstrong W., Shulinq., Liwei H., Ke Z. & Meshnick S. Increase in Prevalence of *Pneumocystis carinii* mutations in patients with AIDS and *P. carinii* pneumonia, in the United States and China. *The Journal of Infectious Diseases*, 2004, *189*, 1684-1687.

Kimchi-Sarfaty C., Oh J.M., Kim I.W., Sauna Z.E., Calcagno A.M., Ambudkar S.V. & Gottesman M.M. A "silent" polymorphism in the MDR1 gene changes substrate specificity. *Science*, 2007, *315*, 525-528.

Klein N.C., Duncanson F.P., Leno, T.H., Forszpaniak C., Sherer C.B., Quentzel H., Nunez M., Suarez M., Kawwaff O., Pitta-Alvarez A. *et al.* Trimethoprim-sulfamethoxazole *versus*

pentamidine for *Pneumocystis carinii* pneumonia in AIDS patients: results of a large prospective randomized treatment trial. *AIDS*, 1992, *6*, 301-305.

Kovacs J.A., Allegra C.J., Beaver J., Boarman D., Lewis M., Parrillo J.E., Chabner B. & Masur H. Characterization of de novo folate synthesis in *Pneumocystis carinii* and Toxoplasma gondii: potential for screening therapeutic agents. *The Journal of Infectious Diseases*, 1989, *160*, 312-320.

Lane B.R., Ast J. C., Hossler P.A., Mindell D.P., Bartlett M.S., Smith J.W. & Meshnick S.R. Dihydropteroate synthase polymorphisms in *Pneumocystis carinii*. *The Journal of Infectious Diseases*, 1997, *175*, 482-485.

Latouche S., Lacube P., Maury E., Bolognini J., Develoux M., Girard P.M., Godet C., Lebrette M.G., Mayaud C., Guillot J. & Roux P. *Pneumocystis jirovecii* dihydropteroate synthase genotypes in French patients with pneumocystosis: a 1998-2001 prospective study. *Medical Mycology*, 2003, *41*, 533-537.

Li K., He A., Cai W.P., Tang X.P., Zheng X.Y., Li Z.Y. & Zhan X.M. Absence of *Pneumocystis jirovecii* dihydropteroate synthase gene mutations among samples from a group of AIDS patients in China. *Scandinavian Journal of Infectious Diseases*. 2009, *41*, 152-154.

Lopez P., Espinosa M., Greenberg B. & Lacks S.A. Sulfonamide resistance in *Streptococcus pneumoniae*: DNA sequence of the gene encoding dihydropteroate synthase and characterization of the enzyme. *Journal of Bacteriology*, 1987, *169*, 4320-4326.

Lundberg B.E., Davidson A.J. & Burman W.J. Epidemiology of *Pneumocystis carinii* pneumonia in an era of effective prophylaxis: the relative contribution of non-adherence and drug failure. *AIDS*, 2000, *14*, 2559-2566.

Ma L, Kovacs J.A., Cargnel A., Valerio A., Fantoni G. & Atzori C. Mutations in the dihydropteroate synthase gene of human-derived *Pneumocystis carinii* isolates from Italy are infrequent but correlate with prior sulfa prophylaxis. *The Journal of Infectious Diseases*, 2002, *185*, 1530-1532.

Ma L., Borio L., Masur H. & Kovacs J.A. *Pneumocystis carinii* dihydropteroate synthase but not dihydrofolate reductase gene mutations correlate with prior trimethoprim-sulfamethoxazole or dapsone use. *The Journal of Infectious Diseases*, 1999, *180*, 1969-1978.

Martin J.N., Rose D.A., Hadley W.K., Perdreau-Remington F., Lam P.K. & Gerberding J.L. Emergence of trimethoprim-sulfamethoxazole resistance in the AIDS era. *The Journal of Infectious Diseases*, 1999, *180*, 1809-1818.

Masur H. Prevention and treatment of pneumocystis pneumonia. *New England Journal of Medicine*, 1992, *327*, 1853-1860.

Matos O., Costa M.C., Correia I., Monteiro P., Vieira J.R., Soares J., Bonnet M., Esteves F. & Antunes F. *Pneumocystis jiroveci* infection in immunocompetent patients with pulmonary disorders, in Portugal. *Acta Médica Portuguesa*, 2006, *19*, 121-126.

Matos O., Costa M.C., Correia I., Monteiro. P., Monteiro M., Soares J., Vieira J.R., Bonnet M. & Antunes F. *Pneumocystis jirovecii* carriage in Portuguese immunocompetent patients: preliminary results. *Journal of Eukaryotic Microbiology*, 2003, *50* (Suppl.), 647-648.

Medrano F.J., Montes-Cano M., Conde M., De La Horra C., Respaldiza N., Gasch A., Perez-Lozano M.J., Varela J.M. & Calderon E.J. *Pneumocystis jirovecii* in general population. *Emerging Infectious Diseases*, 2005, *11*, 245-250.

Mei Q., Gurunathan S., Masur H. & Kovacs J.A. Failure of co-trimoxazole in *Pneumocystis carinii* infection and mutations in dihydropteroate synthase gene. *Lancet*, 1998, *351*, 1631-1632.

Meshnick S.R. & Kazanjian P. Dihydropteroate synthase and cytochrome B: the problem of emerging resistance, *in*: *Pneumocystis* pneumonia, 3rd ed. Walzer P. & Cushion M.T. (eds), Marcel Dekker, New York, 2005, 631-640.

Miller R.F., Lindley A.R., Ambrose H.E., Malin A.S., Wakefield A.E. Genotypes of *Pneumocystis jirovecii* isolates obtained in Harare, Zimbabwe, and London, United Kingdom. *Antimicrobial Agents and Chemotherapy*, 2003, *47*, 3979-3981.

Montes-Cano M.A, De La Horra C., Martin-Juan J., Varela J.M., Torronteras R., Respaldiza N., Medrano F.J., & Calderón E.J. *Pneumocystis jiroveci* genotypes in the Spanish population. *Clinical Infectious Diseases*, 2004, *39*, 123-128.

Moorman A.C., Von Bargen J.C., Palella F.J.& Holmberg S.D. *Pneumocystis carinii* pneumonia incidence and chemoprophylaxis failure in ambulatory HIV-infected patients. HIV Outpatient Study (HOPS) investigators. *Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology*. 1998, *19*, 182-188.

Morris A., Lundgren J.D., Masur H., Walzer P.D., Hanson D.L., Frederick T., Huang L., Beard C.B. & Kaplan J.E. Current epidemiology of *Pneumocystis* pneumonia. *Emerging Infectious Diseases*, 2004, *10*, 1713-1720.

Nahimana A., Rabodonirina M., Helweg-Larsen J., Meneau I., Francioli P., Bille J. & Hauser P.M. Sulfa resistance and dihydropteroate synthase mutants in recurrent *Pneumocystis carinii* pneumonia. *Emerging Infectious Diseases*, 2003, *9*, 864-867.

Navin T.R., Beard C.B., Huang L., Del Rio C., Lee S., Pieniazek N.J., Carter J.L., Le T., Hightower A. & Rimland D. Effect of mutations in *Pneumocystis carinii* dihydropteroate synthase gene on outcome of *P carini*i pneumonia in patients with HIV-1: a prospective study. *Lancet*, 2001, *358*, 545-549.

Palella F.J. Jr., Delaney K.M., Moorman A.C., Loveless M.O., Fuhrer J., Satten G.A., Aschman D.J.& Holmberg S.D. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV outpatient study investigators. *New England Journal of Medicine*, 1998, *338*, 853-860.

Pifer L.L., Hughes W.T., Stagno S. & Woods D. *Pneumocystis carinii* infection: evidence for high prevalence in normal and immunosuppressed children. *Pediatrics*, 1978, *61*, 35-41.

POPPERS D.M. & SCHERL E.J. Prophylaxis against *Pneumocystis* pneumonia in patients with inflammatory bowel disease: toward a standard of care. *Inflammatory Bowel Diseases*, 2008, *14* (1), 106-113.

Redhead S.A., Cushion M.T., Frenkel J.K. & Stringer J.R. *Pneumocystis* and *Trypanosoma cruzi*: nomenclature and typifications. *Journal of Eukaryotic Microbiology*, 2006, *53*, 2-11.

Riebold D. & Reisinger E.C. *Pneumocystis jiroveci* partial DHPS gene for dihydropteroate synthase, isolate 171. NCBI accession number AJ586567, 16.10.2003.

RIEBOLD D., FRITZSCHE C., LADEMANN M., BIER A. & REISINGER E.C. *Pneumocystis jiroveci* dihydropteroate synthase gene mutations at codon 171 but not at codons 55 or 57 detected in Germany. *Clinical Infectious Diseases*, 2006, *42*, 582-583.

RIEBOLD D., MUNK A.K. & REISINGER E.C. *Pneumocystis jiroveci* partial mRNA for dihydropteroate synthase. NCBI accession number AJ746341, 07.06.2004.

ROBBERTS F.J.L., CHALKLEY L.J., WEYER K., GOUSSARD P. & LIEBOWITZ L.D. Dihydropteroate synthase and novel dihydrofolate reductase gene mutations in strains of *Pneumocystis jirovecii* from South Africa. *Journal of Clinical Microbiology*, 2005, *43*, 1443-1444.

SANTOS L.D., LACUBE P., LATOUCHE S., KAC G., MAYAUD C., MARTEAU M., POIROT J.L., MAURY E., GUILLOT J. & ROUX P. Contribution of dihydropteroate synthase gene typing for *Pneumocystis carinii* f. sp. *hominis* epidemiology. *Journal of Eukaryotic Microbiology*, 1999, *46*, 133S-134S.

SEPKOWITZ K.A. Opportunistic infections in patients with and patients without acquired immunodeficiency syndrome. *Clinical Infectious Diseases*, 2002, *34*, 1098-1107 [Erratum, *Clin Infect Dis* 2002, *34*, 1293].

SHEN L.X., BASILION J.P. & STANTON V.P. Jr. Single-nucleotide polymorphisms can cause different structural folds of mRNA. *Proceedings of the National Academy of Sciences of the United States of America*, 1999, *96*, 7871-7876.

SIRIPATTANAPIPONG S., LEELAYOOVA S., MUNGTHIN M., WORAPONG J. & TAN-ARIYA P. Study of DHPS and DHFR genes of *Pneumocystis jirovecii* in Thai HIV-infected patients. *Medical Mycolology*, 2008, *46*, 389-392.

STRINGER J.R., BEARD C.B., MILLER R.F. & WAKEFIELD A.E. A new name (*Pneumocystis jirovecii*) for *Pneumocystis* from humans. *Emerging Infectious Diseases*, 2002, *8*, 891-896.

TAKAHASHI T., HOSOYA N., ENDO T., NAKAMURA T., SAKASHITA H., KIMURA K., OHNISHI K., NAKAMURA Y. & IWAMOTO A. Relationship between mutations in dihydropteroate synthase of *Pneumocystis carinii* f. sp. *hominis* isolates in Japan and resistance to sulfonamide therapy. *Journal of Clinical Microbiology*, 2000, *38*, 3161-3164.

TOTET A., LATOUCHE S., LACUBE P., PAUTARD J.C., JOUNIEAUX V., RACCURT C., ROUX P. & NEVEZ G. *Pneumocystis jirovecii* dihydropteroate synthase genotypes in immunocompetent infants and immunosuppressed adults, Amiens, France. *Emerging Infectious Diseases*, 2004, *10*, 667-673.

TRIGLIA T., WANG P., SIMS P.F., HYDE J.E. & COWMAN A.F. Allelic exchange at the endogenous genomic locus in *Plasmodium falciparum* proves the role of dihydropteroate synthase in sulfadoxine-resistant malaria. *The Embo Journal*, 1998, *17*, 3807-3815.

TYAGI A.K., MIRDHA B.R., GULERIA R., MOHAN A., LUTHRA K. & SINGH U.B. Study of dihydropteroate synthase (DHPS) gene mutations among isolates of *Pneumocystis jiroveci*. *Indian Journal of Medical Research*. 2008, *128*, 734-739.

VALERIO A., TRONCONI E., MAZZA F., FANTONI G., ATZORI C., TARTARONE F., DUCA P. & CARGNEL A. Genotyping of *Pneumocystis jiroveci* pneumonia in Italian AIDS patients. Clinical outcome is influenced by dihydropteroate synthase and not by internal transcribed spacer genotype. *Journal of Acquired Immune Deficiency Syndrome*, 2007, *45*, 521-528.

VAN HAL S.J., GILGADO F., DOYLE T., BARRATT J., STARK D., MEYER W. & HARKNESS J. Clinical significance and phylogenetic relationship of novel Australian *Pneumocystis jirovecii* genotypes. *Journal of Clinical Microbiology*, 2009, *47*, 1818-1823.

VEDANTAM G., GUAY G.G., AUSTRIA N.E., DOKTOR S.Z. & NICHOLS B.P. Characterization of mutations contributing to sulfathiazole resistance in *Escherichia coli*. *Antimicrobial Agents and Chemotherapy*, 1998, *42*, 88-93.

VIDAL S., DE LA HORRA C., MARTÍN J., MONTES-CANO M.A., RODRÍGUEZ E., RESPALDIZA N., RODRÍGUEZ F., VARELA J.M., MEDRANO F.J. & CALDERÓN E.J. *Pneumocystis jirovecii* colonisation in patients with interstitial lung disease. *Clinical Microbiology and Infection*, 2006, *12*, 231-235.

VISCONTI E., ORTONA E., MENCARINI P., MARGUTTI P., MARINACI S., ZOLFO M., SIRACUSANO A. & TAMBURRINI E. Mutations in dihydropteroate synthase gene of *Pneumocystis carinii* in HIV patients with *Pneumocystis carinii* pneumonia. *International Journal of Antimicrobial Agents*, 2001, *18*, 547-551.

VOLPE F., DYER M., SCAIFE J.G., DARBY G., STAMMERS D.K. & DELVES C.J. The multifunctional folic acid synthesis fas gene of *Pneumocystis carinii* appears to encode dihydropteroate synthase and hydroxymethyldihydropterin pyrophosphokinase. *Gene*, 1992, *112*, 213-218.

WAKEFIELD A.E. *Pneumocystis carinii*. *British Medical Bulletin*, 2002, *61*, 175-188.

WALZER P.D., FOY J., STEELE P., KIM C.K., WHITE M., KLEIN R.S., OTTER B.A. & ALLEGRA C. Activities of antifolate, antiviral, and other drugs in an immunosuppressed rat model of *Pneumocystis carinii* pneumonia. *Antimicrobial Agents and Chemotherapy*, 1992, *36*, 1935-1942.

WISSMANN G., ALVAREZ-MARTINEZ M.J., MESNHICK S.R., DIEHL A.R., PROLLA J.C. Absence of dihydropteroate synthase mutations in *Pneumocystis jirovecii* from Brazilian AIDS patients. *Journal of Eukaryotic Microbiology*, 2006, *53*, 305 -307.

WOLFF A.J. & O'DONNELL A.E. Pulmonary manifestations of HIV infection in the era of highly active antiretroviral therapy. *Chest*, 2001, *120*, 1888-1893.

WORMSER G.P. & KEUSCH G.T. Trimethoprim-sulfamethoxazole in the United States. *Annals of Internal Medicine*, 1979, *91*, 420-429.

WORLD HEALTH ORGANISATION. World Health Organization treating 3 million by 2005: making it happen: the WHO strategy: the WHO and UNAIDS global initiative to provide antiretroviral therapy to 3 million people with HIV/AIDS in developing countries by the end of 2005/Treat 3 million by 2005. http://libdoc.who.int/publications/2003/9241591129.pdf. [Accessed 20 January 2010].

ZAR H.J., ALVAREZ-MARTINEZ M.J., HARRISON A. & MESHNICK S.R. Prevalence of dihydropteroate synthase mutants in HIV-infected South African children with *Pneumocystis jiroveci* pneumonia. *Clinical Infectious Diseases*, 2004, *39*, 1047-1051.

ZINGALE A., CARRERA P., LAZZARIN A., SCARPELLINI P. Detection of *Pneumocystis carinii* and characterization of mutations associated with sulfa resistance in bronchoalveolar lavage samples from human immunodeficiency virus-infected subjects. *Journal of Clinical Microbiology*, 2003, *41*, 2709-2712.

Reçu le 27 janvier 2010
Accepté le 18 mars 2010