Dihydropteroate Synthase and Novel Dihydrofolate Reductase Gene Mutations in Strains of Pneumocystis jirovecii from South Africa

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Dihydropteroate synthase (DHPS) gene mutations have raised concerns about emerging sulfonamide resistance in Pneumocystis jirovecii. DHPS and dihydrofolate reductase (DHFR) gene products were amplified in clinical specimens from South African patients. One of 53 DHPS genes sequenced contained the double mutation Thr55Ala Pro57Ser. DHFR gene mutations detected were Ala67Val and the new mutations Arg59Gly and C278T.

Pneumocystis jirovecii is an important cause of community-acquired pneumonia in immunocompromised individuals. In South Africa, as elsewhere in the world, P. jirovecii pneumonia (PeP) accounts for 36 to 49% of admissions for community-acquired pneumonia in human immunodeficiency virus-positive patients (9, 11, 13). The long-term use of cotrimoxazole for PeP prophylaxis and treatment has possibly selected for resistant strains, resulting in both prophylaxis breakthrough cases and treatment failures (2, 3, 5, 16). Two single mutations, Thr55Ala and Pro57Ser, and a combined double mutation, Thr55Ala Pro57Ser, have previously been detected in the dihydropteroate synthase (DHPS) gene (2, 3, 5, 16). Studies have shown that mutations may arise during therapy, suggesting selection for resistance (2, 8, 15). Although polymorphisms in the dihydrofolate reductase (DHFR) gene have been detected, no mutations have been associated with known DHFR enzymatically active sites or with treatment or prophylaxis failure (8, 14).

The aim of this study was to determine the prevalence of DHPS and DHFR gene mutations in South African strains of P. jirovecii. The specimens studied comprised 178 clinical specimens (28 sputum, 125 tracheal aspirate, 18 bronchoalveolar lavage, and 7 fresh lung biopsy specimens) submitted to the Microbiology Laboratory, Tygerberg Hospital (June 2001 to February 2003); 12 lung sections archived at the Pathology Laboratories, Tygerberg Hospital, Western Cape, South Africa; and 663 sputum specimens (2000 to 2003) collected from patients from five provinces in South Africa (KwaZulu Natal, Western Cape, Eastern Cape, North West, and Mpumalanga). Screening for the presence of P. jirovecii was performed by nested PCR of the mitochondrial large-subunit rRNA gene (17). DHPS and DHFR genes were amplified by using the primers and conditions described previously (6, 8). DHPS and DHFR products were purified and subjected to direct sequencing. The sequences obtained were compared to those reported previously: DHPS wild type, GenBank accession no. U66279 (6); DHFR wild type, GenBank accession no. AF090368 (8). For strain typing, amplification of the internal transcribed spacer (ITS) region of the rRNA gene was performed (7), and PCR products were subcloned prior to sequencing (12). The ITS sequences obtained were compared to those of published ITS regions, and type nomenclature was assigned in accordance with Lee et al. as described by Robberts et al. (12).

Screening of samples with mitochondrial large-subunit rRNA primers produced PCR products indicative of P. jirovecii in 85 of 753 specimens. Only 53 DHPS and 27 DHFR genes were successfully amplified. A total of 53 DHPS genes were sequenced, of which 51 were wild type. One DHPS gene contained a synonymous substitution (T200C), and one gene contained the double mutation Thr55Ala Pro57Ser. The gene containing the double mutation Thr55Ala Pro57Ser was detected in a tracheal aspirate from a patient from whom two specimens (a tracheal aspirate and a postmortem lung biopsy) were submitted. The patient had no exposure to sulfonamides prior to this admission, with the tracheal aspirate being collected 1 day and the lung biopsy being collected 19 days after initiation of cotrimoxazole therapy. The double mutation was detected in one of five clones from the tracheal aspirate, while only wild-type sequences were detected in five clones in the postmortem lung biopsy. All of the viral, bacterial, and fungal cultures performed on the above-mentioned specimens were negative. The postmortem findings attributed the patient’s death to ventilation damage. The only ITS genotype demonstrated on the sequencing of five clones from each specimen (a tracheal aspirate and a postmortem lung biopsy) was Eg. The double mutation is thought to signify the emergence of resistance to sulfonamides (8). In the case of the patient from the Western Cape, the mutations did not appear to be associated with long-term prior therapy. Studies in other countries have shown the presence of DHPS mutations in specimens obtained from patients both with (up to 80%) (10) and without (up to 48%) (3) prior exposure to sulfonamides. It has been suggested that the geographic area of residence is an independent predictor of the harboring of DHPS mutations possibly because of local

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sulfonamide-prescribing practices (1). This was evident in a recent study contrasting the high prevalence of mutations in the United States with the very low prevalence in China, where cotrimoxazole is not used as extensively (4). Zar et al. have similarly reported on the low prevalence of DHPS mutations in Western Cape (4 of 30), finding one specimen with a double mutation at codons 55 and 57 and three specimens with single mutations (two with a mutation at codon 55 and one with a mutation at codon 57) (18). These data suggest that selective pressure on local strains may not have reached the levels found in the United States or Europe but rather resemble those in China.

A total of 27 DHFR genes were successfully amplified, and the wild-type gene was demonstrated in 23 specimens. Three specimens from two patients contained a C200T transition mutation (amino acid substitution Arg59Gly) and a C278T transition, the latter being located in the 42-bp intron region of DHFR (GenBank accession no. AJ692154). None of the patients had long-term exposure to cotrimoxazole before developing PCP. The mutated DHFR gene types were obtained from patients within 3 days of initiation of cotrimoxazole therapy. Sequencing of ITS regions of the DHPS and DHFR genes demonstrated that the P. jirovecii isolates harboring the DHFR C200T transitions had ITS genotypes Eg and Eu. Interestingly, ITS type Eu, which to date has only been found in South Africa (12), was associated with the DHFR mutation Ala67Val. Furthermore, the DHFR mutation Arg59Gly and the intron transition mutation C278T have not previously been described. Although alignment of the mutations (not shown) suggests that they are not located in highly conserved regions, the occurrence of these DHFR mutations could have potential in the development of multilocus sequence typing of P. jirovecii.

Of concern for DHPS and DHFR gene mutation and resistance surveillance in South Africa are the low levels of successful amplification with currently available primers. It was seen to be inappropriate when investigating clinical specimens to link DHPS or DHFR types with ITS genotypes since more than one ITS genotype can be obtained from a patient with a single DHPS or DHFR type.

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