

TITLE: NESTED PCR TO DETECTED CO-INFECTION BY *Histoplasma capsulatum* AND *Pneumocystis jirovecii* IN HIV INFECTED PATIENTS WITH PNEUMONIA FROM MAPUTO CENTRAL HOSPITAL, MOZAMBIQUE

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ABSTRACT:

Histoplasma capsulatum is a thermally dimorphic fungus. Histoplasmosis can range in spectrum from a localized pulmonary to a disseminated infection and can affect both immunocompetent and immunocompromised hosts. *Pneumocystis jirovecii* is a fungal pathogen that causes pneumocystosis, a common and often serious opportunistic pulmonary infection in immunocompromised patients. The clinical symptoms of pulmonary histoplasmosis in immunocompromised patients can imitate *P. jirovecii* pneumonia, tuberculosis and other fungal infections. Therefore, a high index of suspicion and a reliable test are needed to make an early differential diagnosis and to initiate the specific therapy of these mycoses. Fifty clinical samples of bronchoalveolar lavage were analyzed from hospitalized or observed patients with HIV infection and pneumonia from Maputo Central Hospital, Mozambique. Genomic DNA was extracted from clinical samples using the QIAamp[®] DNA Mini Kit (QIAGEN[®]) according to the manufacturer's protocol. Nested PCR from 100-kDa-like protein and mtLSUrRNA regions were used to identify the *H. capsulatum* and *P. jirovecii*, respectively. Among the 50 patients, 30 were female and 20 male, with a mean age of 36.38 years old. The 26 samples derived from *Pneumocystis* pneumonia (PCP) study and 24 samples derived from Kaposi Sarcoma study. The majority of patients (n=38) were under HAART. Clinical characteristics and associated risk factors found: smokers (n=10); cough (n=18); fever (n=33); infiltrate (n=46); hypoxemia (n=21); prophylaxis (n=23); dyspnea (n=23); and weight loss (n=38). The Nested PCR revealed 17 samples positive (210 bp) just for *H. capsulatum*, 8 samples positive (290 bp) just for *P. jirovecii*, 11 samples positive for *H. capsulatum* and *P. jirovecii* (co-infection), and 14 samples negative. PCR from β -globin human gene fragment was positive for all samples, confirming the presence of human DNA and absence of PCR inhibitors. Smoke and hypoxemia (p \leq 0.05) were risk factors for PCP as well as the gender for histoplasmosis. The scoring system to diagnose PCP by Smith, Forbes & Gazzard (1992) was used, and 15 patients who presented a probability of having PCP above 80%, 12 were positive in Nested PCR. In conclusion, the Nested PCR technique, in combination with the clinical characteristics and risk factors, was determinant in the definitive diagnosis of pulmonary histoplasmosis and pneumocystosis, mainly in the cases of co-infection.

These authors contributed equally to this work.

Keywords: *Histoplasma capsulatum*, *Pneumocystis jirovecii*, pneumonia, HIV-infection, co-infection

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