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Sporothrix schenckii Sensu Lato identification in fragments of skin lesion cultured in NNN medium for differential diagnosis of cutaneous leishmaniasis

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ABSTRACT

Eighty-nine patients with clinical suspicion of leishmaniasis were referred for differential diagnosis. *Sporothrix schenckii* sensu lato was isolated in Novy-MacNeal-Nicolle + Schneider media in 98% of 64 patients with final diagnosis of sporotrichosis. This medium may be suitable for diagnosis of sporotrichosis in areas where cutaneous leishmaniasis is also endemic.

Cutaneous leishmaniasis (CL) is an infectious disease caused by protozoa of the genus *Leishmania* (World Health Organization, 2010). Sporotrichosis is a subacute or chronic subcutaneous mycosis worldwide distributed but more prevalent in tropical and subtropical areas, caused by the dimorphic fungi *Sporothrix schenckii* sensu lato (Rangel-Gamboa et al., 2016; Chakrabarti et al., 2015). Both are endemic diseases in the State of Rio de Janeiro (RJ). At least 830 cases of sporotrichosis (SES Newsletter, 2014) and 50 cases of CL (Brazilian Ministry of Health, 2016) were reported in RJ between 2013 and 2014.

The most frequent type of CL is the localized form, with single or multiple cutaneous lesions (Brazilian Ministry of Health, 2013; World Health Organization, 2010). The typical ulcer of CL is painless and usually located in areas of skin exposed to insect bites. It has rounded or oval shape, with infiltrated erythematous base and well defined high edges (Pessôa and Barreto, 1948). It may occasionally present with verrucous lesions or with regional lymphadenopathy and nodular lymphangitis (Brazilian Ministry of Health, 2013). The last type (sporotrichoid leishmaniasis) is frequently reported in the Middle East (Cozzani et al., 2011).

Sporotrichosis usually extends along the lymphatic path (lymphocutaneous type) or occasionally remains localized (fixed type). The rarer disseminated presentations are mainly related to immunodeficiencies (De Lima Barros et al., 2011). The lymphocutaneous type starts with an erythematous papule or pustule that evolves into a nodule or plaque tending to ulcerate. Lymphangitis and secondary lesions (nodules or gums) develop, following the path of lymphatic vessels (De Lima Barros et al., 2011; Ramos-e-Silva et al., 2007). The fixed type presents with a single ulcerated or verrucous lesion at the inoculation site, without lymphatic involvement (De Lima Barros et al., 2011).

The similar clinical features in both diseases lead to differential diagnosis (Pavlidakey et al., 2015; López-Escobar et al., 2007; Tobin and Jinh, 2001).

This study included 89 patients with ulcerated skin lesions and a preliminary clinical suspicion of cutaneous leishmaniasis, who had confirmed diagnosis of leishmaniasis or sporotrichosis at Instituto Nacional de Infectologia Evandro Chagas, Fundação Oswaldo Cruz, Rio de Janeiro, between 2013 and 2015.

A biopsy of a skin lesion was performed as part of the routine differential diagnostic procedures. Fragments of the lesions were cultivated in biphasic Novy-MacNeal-Nicolle (NNN) plus Schneider media, supplemented with 10% fetal calf serum and antibiotics (200 U penicillin +100 μg streptomycin) to detect the presence of *Leishmania* protozoa as described by Almeida et al. (2011). Some fragments were also cultivated on 2% Sabouraud dextrose agar (SDA) and Mycosel agar (Myc) media at 25 °C. Filamentous fungi were identified by morphological analysis (macro and micromorphology), and suspected isolates of *Sporothrix schenckii* sensu lato were

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transferred to brain heart infusion (BHI) agar medium at 35 °C for confirmation of fungal dimorphism. Molecular tests for both diseases were not performed in routine procedures, due to their high costs.

Culture for *Leishmania* (NNN + Schneider) were considered positive in microscopic analysis when mobile flagellated parasites with the characteristics of promastigote forms of the protozoa were visualized. Hyphae and conidiae recognized in the microscopic evaluation in NNN + Schneider evidenced fungi.

Twenty patients confirmed *Leishmania* parasites growth in NNN + Schneider media, and five had visualization of amastigotes in direct exam or histopathology. There was no fungi growth in NNN + Schneider nor in SDA/Myc media in the fragments of lesions from CL cases. CL patients were infected in RJ in 48% of the cases, and the remaining were infected in other Brazilian states.

Sporothrix schenckii sensu lato strains were isolated in 2% SDA and Myc media from fragments of cutaneous lesions of 64 patients. The time of growth of fungi in these media was 11 days. *Sporothrix schenckii* sensu lato was also isolated in NNN + Schneider media in 98% of these cases (Fig. 1). The average time of growth of fungi in NNN + Schneider media between 26 and 28 °C was 9 days. In all these cases, the culture of aliquots of NNN + Schneider media on SDA, Myc (Fig. 2), and BHI confirmed the identification of *Sporothrix schenckii* sensu lato. All 64 patients with sporotrichosis were infected in RJ; 67% had lymphocutaneous and 33% had fixed cutaneous ulcerated type.

All 89 patients received specific treatment according to each confirmed disease (De Lima Barros et al., 2011; Schubach et al., 2005) and evolved to complete healing of their respective lesions.

We observed that NNN + Schneider media proved to be suitable for the isolation in culture of both *Leishmania* protozoa and *S. schenckii* sensu lato fungi.

The occurrence of the cutaneous fixed type in one third of the patients with sporotrichosis, with similar characteristics to the most typical CL lesions, demonstrates the similarity of the clinical presentation for both diseases in several cases. Moreover, 52 patients with sporotrichosis and a positive Montenegro skin test were previously reported in RJ (De Lima Barros et al., 2005; Barros et al., 2004). Such findings indicate the need for differential diagnosis between these diseases.

Lymphocutaneous lesions of sporotrichoid leishmaniasis also demand differential diagnosis (Tobin and Jinh, 2001). Although we have not observed in this study patients with sporotrichoid CL, some cases with this atypical presentation were reported in endemic areas (Pavlidakey et al., 2015; Hinojosa et al., 2014; Sampaio et al., 1996).

In 1999, three cases of CL and sporotrichosis co-infection were reported in Colombia (Agudelo et al., 1999). We have not identified cases of such co-infection in a reference center for these infectious diseases in an endemic area for both.

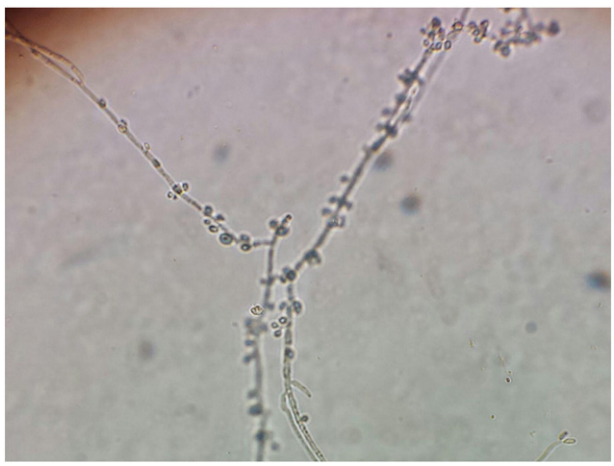

Fig. 1. *Sporothrix schenckii* sensu lato in NNN + Schneider media, magnification 400 X.

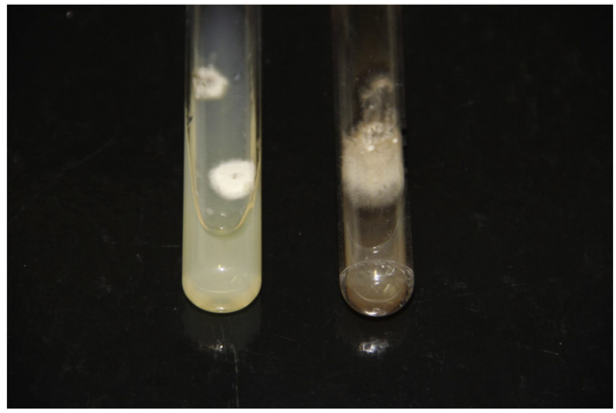

Fig. 2. Aliquots of NNN + Schneider media growth on Sabouraud agar and Mycosel agar.

The growth of other microorganisms in NNN + Schneider media, which is appropriate for the culture of *Leishmania* parasites, may be frequently interpreted as contamination of the media (Chouihi et al., 2009). The search and identification of the contaminating microorganism is not always a routine procedure, and disposal of the material is usually performed.

NNN + Schneider media are more expensive (equivalent to 41 US dollars in June,2016) than classical mycological media (25.3 US dolars), and the simple substitution of these media for those ones is not cost-effective. However, when the clinical signs and symptoms point to both leishmaniasis and sporotrichosis, NNN + Schneider media may be performed first; when there is fungi growth in NNN, fungi cultures should subsequently be performed in traditional mycological media. It would save costs when compared to the performance of both *Leishmania* and mycological cultures in parallel.

Fungi growth in both media evidence hyphae and conidiae. Culture in BHI is necessary in any case to demonstrate fungal dimorphism. However, mycological traditional media are not suitable for *Leishmania* growth. The main differential characteristic in the microscopic analysis regarding both microrganisms is the mobility of *Leishmania*, when compared to the motionless fungi.

The fungal contamination occurring in NNN + Schneider media during the diagnostic procedures should be evaluated for evidence of the occurrence of sporotrichosis, mainly in RJ. We recommend further studies using NNN as an alternative medium to the growth of *Sporothrix schenckii* sensu lato, especially in areas where leishmaniasis and sporotrichosis endemics overlap.

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Conflicts of interest statement

The authors declare that they have no conflicts of interest.

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