TITLE: ANTI-*Trichophyton rubrum* BIOFILM ACTIVITY OF NITRIC OXIDE NANOPARTICLES (NO-np) AND EFINACONAZOLE 10% (Jublia®)

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ABSTRACT:

Biofilm formation is an important virulence factor of certain pathogenic fungi. Over the last few years, filamentous fungi, particularly the dermatophytes *Trichophyton rubrum* and *T. mentagrophytes*, have been recognized as biofilm-forming microorganisms. Therefore, new approaches to overcome this newly identified impediment are needed. Nitric oxide-releasing nanoparticles (NO-np) are currently in development for use against superficial fungal infections and bacterial abscesses, and have shown efficacy against bacterial biolfilms. In this context, this work aimed to evaluate, for the first time, the *in vitro* anti-*T. rubrum* biofilm potential of nitric oxide nanoparticles (NO-np), and compare to a newly FDA approved triazole antifungal efinaconazole 10%. Firstly, the minimal inhibitory concentrations (MIC) and minimal fungicidal concentrations (MFC) of *T. rubrum* ATCC MYA-4438 and a clinical isolate from Montefiore Medical Center, Bronx, NY, USA were determined according to the methods in M-38A2 by the Clinical and Laboratory Standards Institute (CLSI), with minor modifications. After treatment with NO-np and efinaconazole, the metabolic activity of pre-formed biofilms, were determined using the XTT reduction assay. Additionally, scanning electron microscopy (SEM) and confocal microscopy were performed. NO-np and efinaconazole inhibited the *in vitro* growth of *T. rubrum* in planktonic conditions. Under biofilm growth conditions, both the clinical and ATCC isolates formed mature biofilms at 72 hours. The biofilms formed by ATCC strain produced more biomass than the clinical isolate. The clinical isolate biofilms were resistant to efinaconazole while the biofilms formed by the ATCC strain were susceptible ($SMIC_{50}$ = 320 mg/L). The opposite occurred when biofilms were treated with NO-np: the biofilms formed by the clinical isolate were susceptible to NO-np ($SMIC_{50}$ = 40 mg/mL), while the biofilms formed by the ATCC strain were resistant to all concentrations tested. The SEM results of the treated biofilms were consistent with the results shown by the XTT reduction assay. However, both efinaconazole and NO-np reduced the thickness of both biofilms. These results suggest that biofilm formation may explain the resistance of dermatophytes to antifungals and why prolonged treatment that is usually required in onychomycosis. Also, the findings support the concept that biofilms must be considered in when developing and testing new drugs for the treatment of dermatophytosis.

Keywords: *T. rubrum* biofilms, efinaconazole, nitric oxide nanoparticles, resistance, susceptibility

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