Antifungal Efficacy of Amphotericin B against Dermatophytes and its Relevance in Recalcitrant Dermatophytoses: A Commentary

Dermatophytoses are the most common fungal infections worldwide, and are especially frequent in tropical and subtropical regions due to the high temperature and relative humidity.[1] The most common clinical form of dermatophytosis in Indian studies is tinea corporis followed by tinea cruris.[2] Tinea corporis *et* cruris is the most common mixed clinical type.[2]

Dermatophytes occupy three ecological niches – anthropophilic (species found only on humans – *Trichophyton rubrum*, *T. tonsurans*), zoophilic (found on other animals too – *Microsporum canis*, *T. equinum*, *T. verrucosum*), and geophilic (found on soil and only occasionally infecting humans and other animals – *Microsporum gypseum*). Overall, in majority of the studies, genus *Trichophyton* dominates with 90% clinical isolates followed by *Epidermophyton* (5%) and *Microsporum* (5%). *T. rubrum* is the most common dermatophyte worldwide.[3] In most studies from India too, *T. rubrum* followed by *T, mentagrophytes* are the commonest dermatophytes isolated from clinical strains.[2]

Conventionally, localized dermatophyte infections are amenable to topical treatment modalities with a few exceptions, including tinea capitis, onychomycosis, tinea of more than one body region simultaneously, extensive tinea corporis, and extensive/bullous tinea pedis, where systemic antifungals would be preferable to topical drugs, either alone or in combination with topical antifungals. In areas where recalcitrant infections abound, there has been an urge to use novel antifungals based on the, yet unproven, premise of "resistance," based on microbiological data, which itself is woefully inadequate. Resistance can be clinical or microbiological. The former is a failure of therapy due to sub-therapeutic drug levels at the site of the infection due to various causes, including certain pharmacokinetics of the drug, drug interactions, poor patient compliance, overwhelming infection, difficult-to-reach site of infection, and altered immune status of the host.[4,5] Microbiological resistance could be due to a failure of drug to suppress growth of the test organism under certain growth conditions; however, it is consistently a poor predictor of clinical outcome due to lack of accurate correlation between *in vitro* testing and *in vivo* outcomes and also because the host immune response has a predominant role in disease resolution.

There are a handful of reports of clinical failure or relapse (within 4 weeks of stopping therapy) that have been published with documented antifungal drug resistance. Mukherjee *et al.*[6] published the first confirmed report of terbinafine resistance in dermatophytes in 2003, wherein six isolates of *T. rubrum* from a single onychomycosis patient were found to be resistant to terbinafine. Favre *et al.*[7] and later Osborne *et al.*[8] further researched the same isolates and concluded that the resistance appeared to be due to a single amino acid substitution in the squalene epoxidase gene. Usual minimum inhibitory concentrations (MICs) in susceptible isolates of *T. rubrum* were 0.03 μg/ml versus >1.0 μg/ml (4000 × higher) in the resistant isolates.[6] Osborne *et al.*[9] and, more recently, Digby *et al.*[10] have reported two more documented cases of terbinafine-resistant *T. rubrum*.

Though in India conventional topical agents used include azoles, terbinafine, ciclopirox, and amorolfine, a perceived "clinical" resistance in recalcitrant cases has prompted clinicians to attempt the use of drugs like amphotericin B (AMB).

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AMB is available in topical lipid-based formulations for optimal permeation through the stratum corneum. Its use in dermatophytic infections is an off-label [non-Food and Drug Administration (FDA) approved] indication.

AMB is a broad spectrum antifungal drug that has been used parenterally for many years. It remains the "gold standard" for treatment of disseminated invasive mycoses.[11] It is fungicidal primarily because of its unique structure characterized by both hydrophilic (polyhydroxyl) and hydrophobic (polyene) faces on its long axis.[12] AMB binds to ergosterol, forming pores that cause rapid leakage of monovalent ions (K^+, Na^+, H^+, and Cl^-) and subsequent fungal cell death. Its poor bioavailability and adverse effects through parenteral use have prompted development of phospholipid-based formulations that is safer with higher bioavailability. Topical AMB has been studied in cutaneous candidiasis and nondermatophyte mold (NDM) infections and found to be efficacious, both *in vitro* and *in vivo*.[13-16] A literature search revealed no clinical studies on the use of AMB *in vivo* in dermatophyte infections, but a handful of studies have been published documenting the *in vitro* susceptibility testing of dermatophytes to AMB.[17-21] Considering the favorable results *in vitro* against dermatophytes and its clinical efficacy in candidiasis and NDM infections, it is logical to expect clinical efficacy of topical AMB in dermatophytic infections. Yenisehirli *et al*.[17] studied the *in vitro* activity of six antifungals against dermatophytes [Table 1]. They compared the MIC ranges, MIC_{50}, MIC_{90}, mean MIC, and geometric mean (GM) MIC values of terbinafine, AMB, itraconazole, miconazole, ketoconazole, and griseofulvin for 177 clinical isolates. Terbinafine was found to be the most effective drug ($P < 0.05$). AMB was more effective than the other four drugs against *T. rubrum* and *T. verrucosum*. Against *T. mentagrophytes* and *Epidermophyton floccosum*, AMB was found to be better than other drugs but was inferior to terbinafine and itraconazole. Aktas *et al*. compared five antifungal drugs against dermatophytes using the E-test method. They found that caspofungin and itraconazole were the most effective drugs and that AMB was consistently better than ketoconazole and fluconazole against all the dermatophytes tested.[18] Fernandez-Torres *et al*.[19] too compared 10 antifungal drugs against 508 dermatophyte strains and found AMB to be superior to fluconazole. Coelho *et al*.[20] compared the *in vitro* antifungal susceptibility of the microconidia of *Trichophyton rubrum* and *T. tonsurans* to 5 commonly used drugs- AMB, fluconazole, terbinafine, itraconazole and griseofulvin. They found AMB to be the most superior drug. The MIC were least for AMB (AMB < TF < ITZ < GF < FCZ for *T. rubrum* and AMB < TF < ITZ < GF < FCZ for *T. tonsurans*) [Table 2]. Badali *et al*.[21] evaluated efficacy of nine antifungals (AMB, fluconazole, itraconazole, voriconazole, posaconazole, isavuconazole, caspofungin, anidulafungin, and terbinafine). The most effective drug was terbinafine followed by anidulafungin followed by AMB (*T. mentagrophytes* and *T schoenleinii*).

Table 1: *In vitro* antifungal susceptibility of common clinical dermatophyte isolates against six drugs – terbinafine, amphotericin B, miconazole, itraconazole, ketoconazole, and griseofulvin[17]

Isolates	Geometric Mean of MIC
T. rubrum (*n*=78)	TF < AMB < GF < MCZ = ITZ < KTZ
T. mentagrophytes (*n*=49)	TD < ITZ < AMB < MCZ < KTZ < GF
E. floccosum (*n*=30)	TF < ITZ < AMB < MCZ < GF < KTZ
T. verrucosum (*n*=16)	TF < AMB < GF < MCZ < ITZ < KTZ
T. tonsurans (*n*=4)	TF < AMB < MCZ < ITZ < KTZ < GF

TF: Terbinafine, AMB: Amphotericin B, GF: Griseofulvin, MCZ: Miconazole, ITZ: Itraconazole, KTZ: Ketoconazole

Table 2: *In vitro* antifungal susceptibility of microconidia of Trichophyton to five common antifungals – terbinafine, fluconazole, griseofulvin, itraconazole, and amphotericin B

Isolates	MIC (mg/L)
T. rubrum	AMB < TF < ITZ < GF < FCZ
T. tonsurans	AMB < TF < ITZ < GF < FCZ

AMB: Amphotericin B, TF: Terbinafine, ITZ: Itraconazole, GF: Griseofulvin, FCZ: Fluconazole

It was, however, inferior to other drugs (except fluconazole) against *T. rubrum*, *T. verrucosum*, and *T. violaceum*. Here it is crucial to appreciate that there are no interpretive criteria for AMB versus yeasts or molds. An MIC of >1 µg/ml is often considered as indicative of yeast resistance, but such an interpretive cutoff has not been arrived at for dermatophytes.[22,23]

The clinical applicability of these data has to be weighed rationally, as *in vitro* susceptibility may not always translate into *in vivo* efficacy. The data provided by standard antifungal susceptibility test methods, the MIC, or the disk zone diameter may not always have clinical relevance in the care of patients with fungal infections.[24] Thus *in vitro* data should be interpreted with caution as in dermatophytes a multitude of factors related to the host (immune response, underlying illness, site of infection), the infecting organism (virulence), and the antifungal agent (dose, pharmacokinetics, pharmacodynamics, drug interactions) may be more important than susceptibility test results in determining clinical outcomes for infected patients. Thus *in vitro* susceptibility of an organism to an antifungal agent does not consistently predict a successful therapeutic outcome.[25]

A pertinent and often glossed over fact is that there are different morphological forms of the dermatophytes *in vitro* and *in vivo*. *In vitro*, they mostly exist as microconidia, which are formed from the ends of conidiophores extending laterally from hyphae.[20] However, *in vivo*, dermatophytes often produce arthroconidia, a dormant, hardy, more resistant, spherical spore formed by the fragmentation of hyphae. This change is dependent on local environment changes brought about by the associated

hyperkeratosis and scaling (leads to low local O_2 and high local CO_2). Consequently, arthroconidia are more resistant than microconidia, and thus the *in vitro* efficacy might not always be reproduced in the clinical scenarios unless the arthroconidia are tested *in vivo*.[20]

Thus, it is pertinent to examine the use of AMB in dermatophytic infections clinically. Although the efficacy is probably species-dependent, but a summary of the data shows that AMB is second only to terbinafine and echinocandins (and superior to most azoles) against the most common species, i.e., *T. rubrum* and *T. mentagrophytes*.[17,21] Against *T. schoenleinii* and *T. verrucosum* too, AMB is comparable to itraconazole, while against other species it may be less effective than other topical drugs.[17] Hence, while topical AMB is not superior to terbinafine against dermatophytes, it may, at least in part, provide the answer to the vexing issue of "recalcitrant" infections. However, we must not forget that AMB is the drug of choice for many invasive life-threatening fungal infections and hence it may be prudent to restrict its use to specific cases where its use can be fully justified.

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Conflicts of interest

There are no conflicts of interest.

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