



Antifungal resistance in dermatophytosis: A global health concern

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Abstract

Dermatophytosis is a common dermatological problem in animals as well as humans which is associated with interference in immune function. Unlike the antibacterial resistance which is frequently reported, antifungal resistance is less commonly reported, but there are reports of emerging antifungal resistance in humans and animals. The problem of antifungal resistance can be more severe in comparison to any other drug resistance due to the limited number of antifungals available for therapeutic purposes. Several mechanisms have been put forward to explain the phenomenon of antifungal drug resistance, such as drug efflux by fungal cells, drug detoxification by fungal cells and resistance imposed by structural elements of the fungal cell, target gene mutations, etc. Currently, only three types of antifungal drugs are available – azoles, polyenes, and allylamines; therefore it is mandatory to use the antifungals rationally to contain the problem of rising antifungal resistance. To counter the problem of antifungal resistance indiscriminate over the counter use of antifungal drugs in the treatment of dermatophytosis need to be strongly discouraged. Furthermore, at the research level, whole-genome sequencing of dermatophytes from around the world will aid in a better understanding of fungal pathophysiology and associated drug resistance, potentially leading to new approaches to overcome antifungal resistance. And, lastly, the use of combination therapy offers an advantage of synergistic action of different antifungals with enhanced spectrum activity which could play an instrumental role in reducing the antifungal resistance.

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1. Introduction

Intrinsic features, structures, or modifications thereof which enable fungi to survive in the environment containing antifungal agents constitute the antifungal resistance (Garcia-Rubio et al. 2018) and fungal infections, such as dermatophytosis, do not respond to antifungal therapy. The repercussions of antifungal resistance will be more destructive than the antibiotic resistance because of the limited availability of antifungal drugs. At present only three types of antifungal drugs are available – azoles, polyenes, and allylamines; therefore antifungal resistance will severely narrow down the treatment options available in humans and animals (CDC 2019). Dermatophytosis is a disease of keratinized tissues such as hair, skin, and nails/hoofs in animals and humans caused by a group of pathogenic keratinolytic filamentous fungi known as dermatophytes (Begum et al. 2020). Dermatophytes are the most frequent pathogenic fungi that cause surface mycoses in

humans and animals (Bontem et al. 2020), and dermatophytosis affects roughly 20-25 per cent of the global human population (Dabas et al. 2017). In animals, dermatophytosis is characterized by round focal areas of alopecia, called ringworm and in humans, it is called tinea which is manifested in different forms depending on the body part involved in the infection (Esch et al. 2014). It is associated with significant morbidity and socioeconomic trauma to humans; and economic losses in animals as well.

Taxonomically dermatophytes are grouped under the order Onygenales and the family *Arthrodermataceae* which comprises of 7 genera. However, only three genera – *Microsporum*, *Trichophyton*, and *Epidermophyton* are commonly associated with dermatophytosis in humans and animals (Khurana et al. 2019). Though *Trichophyton rubrum* is the most common causative species of dermatophytosis in humans, the incidence of infections by *T. interdigitale* and *T.*

mentagrophytes are showing an increasing trend (Singh et al. 2018; Rudramurthy et al. 2018; Dabas et al. 2017). However, globally a worrisome aspect of dermatophytosis is the emergence of recalcitrant infections over the past few years. Thus, there is a need for a deeper understanding of pharmacokinetics and pharmacodynamics of the limited antifungal drugs available for the treatment of dermatophytosis in animals and humans, along with the understanding of antifungal resistance and its contributing factors (Khurana et al. 2019).

2. Antifungal resistance and limitations in dermatophyte treatments

The antimicrobial drug resistance is an inevitable evolutionary process of the microbial world. Though fungal resistance is not at par with bacterial resistance, the economic repercussions of fungal infections are highly deplorable (Srinivasan et al. 2014). Because of the limited availability of antifungal drugs reducing the incidence of antifungal resistance is vital for successful antifungal therapy (Sanglard and Odds 2002). Over the past few decades, significant improvements in antifungal therapies have been achieved, but the rise of antifungal resistance in clinical settings because of irrational use (Sultana and Wahiduzzaman 2018) represents a major clinical challenge in treating fungal infections in both human and animals (Wiederhold 2017) which complicates the dermatophyte management in patients (Pfaller 2012).

This antifungal resistance is commonly discussed under two headings – microbiological and clinical. Microbiological resistance is the inability of an antifungal drug to inhibit the growth of pathogenic fungi at normal MIC of the drug or when the inhibition occurs at a concentration which exceeds the MIC of the drug required for wild-type strains (Pfaller 2012). It can be either primary or secondary in nature. When an organism is resistant to a drug without exposure it is called primary or intrinsic microbiological resistance and when it is developed in response to drug exposure it is called secondary or acquired microbiological resistance (Garcia-Rubio et al. 2018). Secondary microbiological resistance is usually driven by altered gene expression (Kanafani and Perfect 2008). Clinical resistance, on the other hand, is described as a circumstance in which an antimicrobial agent fails to suppress an infecting organism despite its susceptibility *in vitro*, resulting in treatment failure. In other words, it's a condition in which a regular dose of antifungal medicine fails to achieve an MIC for the infecting organism at the site of infection, and the host immune system is unable to destroy the infecting organism (Pfaller 2012; Kanafani and Perfect 2008). Furthermore, antifungal drug pharmacokinetics and pharmacodynamics significantly impact the development of antifungal resistance development in the pathogenic fungi/dermatophytes (Nucci and Perfect 2008).

The degree of infection by dermatophytes depends upon

the immunity of animals and humans. The immunocompetent animals/humans mostly develop superficial infections only and immunocompromised animals/humans may develop deep-seated systemic infections. In general, topical dermatophytosis treatment is ineffective in treating certain types of dermatophytosis, such as onychomycosis, since antifungals cannot enter the nail unit and hence cannot entirely eradicate the infection. Another problem with antifungal agents is their interaction with other medications which either hinders the success of therapy or produces harmful outcomes. Hepatotoxicity is also a concern of antifungal therapy against dermatophytes, such as ketoconazole therapy. Also, the recalcitrant dermatophyte infections are common because of limited drug delivery to the site of infection which is mostly because of the avascular and keratinized nature of infected sites.

3. Mechanisms of resistance to antifungal drugs

From the clinical perspective, antifungal resistance has been defined as the persistence of infection/symptoms even after an appropriate antifungal therapy which eventually leads to failure of elimination of fungal infection (Martinez-Rossi et al. 2018). Several molecular mechanisms of antifungal resistance against different antifungal agents have been explored to a considerable extent for certain pathogenic genera of fungi, however, the understanding of antifungal resistance in dermatophytes is poorly characterized (Gnat et al. 2020). From the existing literature most common mechanisms proposed for dermatophyte resistance to antifungal drugs are:

3.1 Drug efflux

Pumping out of antifungal drugs by effluent pumps present in the cell membrane can be one of the mechanisms responsible for the display of antifungal resistance in dermatophytes (Gnat et al. 2020; Martinez-Rossi et al. 2018; El-Awady et al. 2016). An increase in the expression levels of these molecular pumps reduces the intracellular concentration of the antifungal drugs which results in treatment failure because of subtherapeutic drug level and in turn resistance is developed (El-Awady et al. 2016).

3.2 Drug detoxification

At sub-inhibitory levels of antifungal drugs, there occurs enhanced expression of genes involved in cellular detoxification of dermatophytes which contributes to antifungal resistance (Martinez-Rossi et al. 2018; Persinoti et al. 2014). The exposure of *T. rubrum* to acriflavine undecylenic acid induced upregulation of cell antioxidant genes which enhanced its survival against these antifungals (Persinoti et al. 2014; Martinez-Rossi et al. 2018). This detoxification ability of dermatophytes due to enhanced expression of antioxidant enzymes has been correlated with their pathogenicity (Gnat et al. 2018; Martinez-Rossi et al. 2018).

3.3 Heat shock protein activity

Heat shock proteins (HSP) are the chaperones of diverse biological activities and are present in all organisms which help them withstand the stressful conditions, such as exposure of dermatophytes to antifungal drugs (Tiwari et al. 2015; Tamayo et al. 2013). The exposure of dermatophytes to terbinafine and acyclovir at sub-therapeutic levels upregulated the expression of the HSP-70 family, whereas exposure to itraconazole and amphotericin B upregulated the expression of proteins belonging to the small HSP family (Jacob et al. 2015; Martinez-Rossi et al. 2016).

3.4 Structural elements of the cell

The fungal cell structure represents another potential mechanism of antifungal resistance in dermatophytes (Gnat et al. 2020; Martinez-Rossi et al. 2018). The formation of biofilms is one of the modifications in the cell structure of fungi which confers them with the property of antifungal drug resistance and develops a persistent infection as well (Brilhante et al. 2017; Gupta et al. 2016). Recalcitrant dermatophytes have been linked to the formation of biofilms, which serve as a source of prolonged infection and antifungal resistance (Gupta et al. 2016). Furthermore, the keratinized surface structure in dermatophytes reduces the efficiency of antifungal drugs to a considerable extent (Monod et al. 2019).

3.5 Mutations in the enzyme target genes

The mutations in the target genes of several antifungal agents have been reported which renders antifungal therapy useless (Martinez-Rossi et al. 2018). For example, squalene epoxidase, the most common target enzyme of many antifungal drugs, catalyzes the biosynthesis of ergosterol and mutations in the squalene epoxidase results in structural changes which render the antifungal drugs inefficient against this target (Lana et al. 2018; Martinez-Rossi et al. 2018). However, such structural changes do not affect the enzyme function (Martinez-Rossi et al. 2008).

4. Resistance in dermatophytes

Though the antifungal resistance in dermatophytes has not been studied much, there are recent reports which suggest the rising incidence of antifungal resistance (Singh et al. 2018; Rudramurthy et al. 2018). The emergence of recalcitrant dermatophyte infections after completion of recommended antifungal therapy is well established now against griseofulvin in case of *T. rubrum* and *T. tonsurans* infections (Dogra et al. 2019). Because griseofulvin treatment failed, allylamines have become the primary treatment choice for dermatophyte infections (Newland and Abdel-Rahman 2009). In dermatophyte isolates, an amino acid alteration at one of the four locations of the squalene epoxidase protein (Leu 393, Phe 397, Phe 415, His 440) resulted in a higher MIC value against terbinafine (Yamada et al. 2017). On a similar line, in a study from India, about 17% of *T. interdigitale* and 14.3% of *T. rubrum* isolates exhibited high terbinafine resistance

(Rudramurthy et al. 2018). The prolonged exposure to azoles, amorolfine, and terbinafine at a sub-therapeutic level has been ascribed to the development of resistant dermatophytes which have led to treatment failure and in turn persistence of the infections (Ghelardi et al. 2014). Since the susceptibility pattern of different species of dermatophytes towards different antifungals may be different (Bhatia and Sharma 2015), the *in vitro* testing of dermatophyte susceptibility will not only help us understand the epidemiological pattern of antifungal resistance but also may offer help in prescribing an appropriate antifungal drug at the right dose (Verma and Madhu 2017).

5. Prevention or control of antifungal resistance

The following measures can be adopted to control the development of antifungal resistance (Nigam 2015; Shivanna and Inamadhar 2017) :

- a. Judicious use of antifungal agents
- b. Avoiding treatment with sub-therapeutic doses
- c. Use of combination therapy with drugs having different mechanisms of action
- d. Surveillance studies to identify the true nature of antifungal resistance to new drug targets have been identified
- e. New drugs should be directed against the cellular targets which are essential and conserved with no counterpart available
- f. The immunosuppressive drugs such as cyclosporine and D-octapeptides have been found to counteract drug resistance due to efflux pumps
- g. In immunocompromised patients, antifungal agents can be combined with cytokines
- h. Physical modalities of treatment such as photodynamic therapy, lasers, and iontophoresis can also be combined to improve the penetration of antifungal agents, especially in onychomycosis
- i. Advancements in diagnostic testing to inform antifungal treatment and stewardship actions
- j. Reduction of selective pressure and the impact of resistant fungal pathogens
- k. Increased use of antifungals with lower acquisition costs
- l. Development of a novel delivery system for topical antifungal therapy (such as nanoparticles, liposomes, microemulsions, micelles, etc.)

6. Conclusions

Indiscriminate over the counter use of antifungal drugs in the treatment of dermatophytosis has hastened the rise of antifungal resistance. Antifungal resistance can be microbiological, clinical or both. Microbiological antifungal resistance is determined by the factors associated with the

infecting organism which is generally established due to genetic alteration. Clinical antifungal resistance, on the other hand, is linked to host or drug-related factors. These factors may act individually or complement each other to develop strong resistance. It is well established now that commonly used antifungal agents, such as azoles and terbinafine, against dermatophytes are potential resistance inducers. However, globally whole-genome sequencing of dermatophytes will help researchers better understand fungal pathogenesis and associated drug resistance, potentially leading to new approaches to combat antifungal resistance. Further, the use of combination therapy offers an advantage of synergistic action of different antifungals with enhanced spectrum activity which could play an instrumental role in reducing the antifungal resistance.

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