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Review

Antifungal resistance and clinical significance in small animals

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Abstract

Fungal diseases have risen in conjunction with the increasing number of immunosuppressive diseases in human and animal populations. Despite the introduction of new antifungal agents in recent years, the incidence of fungal infections continues to increase, and subsequently, the prevalence of resistance to these drugs is remarkably increased, posing significant health concerns. While antifungal drug resistance is of great importance in human medicine, especially against *Candida* spp., there are few studies about antifungal resistance in veterinary medicine. Indeed, several fungal infections include blastomycosis, candidiasis, coccidiomycosis, cryptococcosis, dermatophytosis, histoplasmosis, and *Malassezia* spp. infections have been reported in dogs and cats. Several antifungal drugs such as polyenes, azoles, pyrimidines, echinocandins, and allylamines have been encountered in feline and canine medicine. However, the desired success could not be obtained from the treatments applied in various cases in recent years due to antifungal resistance. This review aimed to emphasize the main common fungal infections in dogs and cats and the role of developing resistance against antifungal agents on treatment failures. Additionally, we discussed the mode of action of antifungal drugs, mechanisms of resistance, and factors that contribute to the emergence of resistance. In this context, monitoring of antifungal resistance in veterinary clinics and animal facilities by veterinarians and other animal health authorities is recommended.

Keywords: Antifungal resistance, Fungal infection, Antimycotic, Veterinary clinics

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Introduction

Fungal infections are classified into superficial and deep mycoses. Superficial mycoses are limited to the skin and include dermatophytosis and *Malassezia* infections. In general, deep mycoses can be developed in dogs and cats at any age, breed, and sex, but genetic factors could affect the susceptibility to fungal infection (Seyedmousavi et al., 2017).

Blastomycosis (*Blastomyces dermatitidis*), histoplasmosis (*Histoplasma capsulatum*), coccidiomycosis (*Coccidioides immitis*), and cryptococcosis (*Cryptococcus neoformans* and *C. gattii*) are the most common systemic mycoses in dogs (Yıldız et al., 2016). The definitive diagnosis of mycotic infections is based on clinical signs, histopathology, fungal culture, antibody or antigen testing, and real-time polymerase chain reaction (PCR) assays (Seyedmousavi et al., 2018).

Resistance to antimicrobial agents in both animals and humans has important effects on morbidity, mortality, and health costs. It has gained importance in veterinary practice in recent years due to the increase in the incidence of antifungal resistance, which occurred as a result of widespread use of broad-spectrum antifungal compounds, increased incidence of fungal infections, increase in the incidence of viral infections, and the use of immunosuppressive drugs (Bhanderi et al., 2009; Seyedmousavi et al., 2018). In this review, we will discuss the most common fungal infections in dogs and cats and the development of antifungal resistance in animals, which might be a reason for the therapeutic failure of fungal diseases in small animals and could significantly impact the public health.

Common fungal diseases in small animals

Several fungal diseases have been identified in dogs and cats, causing superficial or systemic infections.

Dermatophytosis

Dermatophytosis, colloquially known as ringworm, is the most common fungal disease in dogs and cats. Three genera of dermatophytes, namely *Trichophyton*, *Epidermophyton* and *Microsporum*, are known. It was

found that *M. canis* is the most common dermatophyte isolated from dogs and cats (77.7%), followed by *M. gypseum*, and *Trichophyton terrestre* (Cafarchia et al., 2004). The prevalence of *M. canis* is significantly higher in young dogs and cats than in older animals.

The disease is characterized by alopecia, scaling, crusting, papular and pustular lesions, follicular plugging, erythema, hyperpigmentation, and dystrophic nail growth. Dermatophytosis has a zoonotic importance, especially in immunocompromised persons. Ketoconazole (KTZ) and itraconazole (ITZ) are used to treat *M. canis* and *T. mentagrophytes* in dogs and cats (Swales, 2021). While the development of resistance against dermatophytes is uncommon, it was reported in *T. rubrum*, *T. mentagrophytes*, and *M. canis* (Aneke et al., 2018). Debnath and others isolated dermatophytes from healthy companion animals in India. The authors found that KTZ, ITZ, and amphotericin-B (Am-B) exhibited the lowest minimum inhibitory concentrations (MIC) against *M. canis*, *T. mentagrophytes*, and *M. gypseum*, respectively, while fluconazole (FCZ) and miconazole (MCZ) showed the highest MIC value against these isolates (Debnath et al., 2016).

Malassezia spp.

Malassezia spp., opportunistic lipophilic yeasts in dogs and cats, cause dermatitis or otitis in dogs and cats (Nasiboglu and Or, 2017; Moraru et al., 2019; Swales, 2021). *Malassezia pachydermatis* is the most common species associated with ceruminous otitis externa and a "seborrhoeic" dermatitis in dogs and cats (Guillot and Bond, 2020). Some breeds, such as Sphynx cats and Basset Hounds dogs, are predisposed to having higher numbers of *Malassezia* spp. on their skin and ears (Swales, 2021).

Topical or systemic azole therapy is usually used to treat *Malassezia* infections. MCZ, ITZ or KTZ are the most effective drugs. Recent studies have shown that most wild-type *Malassezia* spp. are sensitive to commonly used azoles such as KTZ, ITZ, and MKZ, although the effect of FCZ is more variable (Brilhante et al., 2018; Schlemmer et al., 2019). In laboratory studies, the development of resistance against azoles by *M. pachydermatis* was reported (Nakano et al., 2005; Jesus et al., 2011).

Candidiasis

Candida spp., a saprophytic yeast commonly found in various animal species, is normally found on animals' skin, digestive tract, upper respiratory tract, and genital mucosa. Local infections of the skin (outer ear, perineum, nail folds), oral mucosa, cornea, urinary tract, and gastrointestinal tract infections have been reported (Pressler et al., 2003).

This opportunistic pathogen can cause localized infection in immunosuppressed patients, mainly in patients with long-term corticosteroid or antimicrobial therapy, cytotoxic chemotherapy, and patients suffering from diabetes mellitus (Klepser et al., 1997). In addition, neutropenia associated with parvoviral enteritis and cyclophosphamide usage is a predisposing factor for candida infections (Willems et al., 2017). In cats, candidiasis is rare and can cause dermatitis similar to *Malassezia* spp. ITZ and Am-B lipid complex are considered the treatments of choice in dogs. Pressler and others detected two resistant *Candida* spp. strains to KTZ, ITZ, and FCZ in urine samples collected from cats and dogs suffering from urinary tract infections (Pressler et al., 2003).

Aspergillosis

Aspergillosis is a mycotic disease of various animal and human hosts, especially in individuals suffering from immunosuppression. It causes keratomycosis, fungal otitis externa, sinonasal aspergillosis (SNA), sino-orbital aspergillosis, bronchopulmonary and diffuse aspergillosis. SNA is very common in large and non-brachycephalic breed dogs and is caused by the *A. fumigatus* complex. It is rare in cats, but diabetes mellitus can be diagnosed in some affected cats (Leite-Filho et al., 2016). Disseminated aspergillosis in dogs is thought to result from genetic immunodeficiency. Young female German Shepherds are predisposed, and affected dogs have a history of immunosuppressive medication (Renschler, 2017). Resistance to azoles has been reported in *Aspergillus* spp. isolated from cats and dogs (Barachetti et al., 2009; Barrs and Talbot, 2014; Talbot et al., 2015).

Cryptococcosis

Cryptococcosis is an opportunistic fungal disease caused by encapsulated yeast species, as a multisystemic disease of various species, including dogs, cats, and humans (Vorathavorn et al., 2013). *C. neoformans* and *C. gattii* are the two main pathogenic species (Vorathavorn et al., 2013). Cryptococcosis is more common in dogs than in cats (Duncan et al., 2006; Trivedi et al., 2011). In dogs, the organism generally affects immune-competent hosts and often causes disseminated disease with multi-organ involvement (Duncan et al., 2006; Vorathavorn et al., 2013). The disease is characterized by the central nervous system (CNS) involvement in dogs (Trivedi et al., 2011). Azole and Am-B are the most effective drugs against cryptococcosis in dogs; however, cryptococcus is resistant to antifungal drugs, especially FCZ. Additionally, the altered mental status in dogs with CNS cryptococcosis is a negative prognostic indicator (Vorathavorn et al., 2013).

Blastomycosis

Blastomycosis, caused by *B. dermatitidis*, is a systemic fungal disease in dogs, cats, and humans. Dogs are infected by inhalation of fungal spores from soil or decaying matter, and the disease is characterized by respiratory manifestations, lethargy, inappetence, pyrexia, lameness, blindness, and neurological signs (Brömel and Sykes, 2005). Young males of the large breed hunting dogs are typically reported as the group at the highest risk for infection with *B. dermatitidis*. Am-B, KTZ, and ITZ could be used to treat blastomycosis (Legendre et al., 1996). However, KTZ is not effective compared with ITZ and Am-B.

Histoplasmosis

Histoplasmosis is a zoonotic systemic mycosis caused by *H. capsulatum*. Infection occurs by inhalation of *Histoplasma* spores or ingestion and infection via open wounds (Džaja et al., 2004; Canteros et al., 2010). The disease is more severe in the immunocompromised hosts (Greene, 2011). Clinically, the disease is characterized by gastrointestinal problems (diarrhea), respiratory manifestations (sneezing), skin and gingival lesions (multiple crusted papules located over lips, back of head, neck, thorax, and lumbar region), swelling of lymph nodes (submandibular, popliteal), and thrombocytopenia (Ortiz-Yépez et al., 2015). ITZ and KTZ can be used for the treatment of histoplasmosis (Brömel and Sykes, 2005). However, the use of KTZ, in this case, proved to be an adequate treatment option (Ortiz-Yépez et al., 2015).

Coccidiomycosis

Coccidioidomycosis in dogs and cats, caused by *C. immitis* and *C. posadasii*, is characterized by pulmonary infection. In dogs, other signs such as lameness (due to osteomyelitis), lymphadenopathy, CNS dissemination (seizures, ataxia, behavioral changes, and coma), cardiac involvement (heart failure, arrhythmia, syncope, and sudden death) are also common (Johnson et al., 2003; Shubitz, 2007). Also, in cats, several signs of dissemination such as skin lesions, abscesses, subcutaneous masses, lymphadenopathy, lameness, and neurological and ocular abnormalities were reported (Greene and Troy, 1995). Azoles and Am-B are effective for treating coccidioidomycosis but must be given intravenously. Lipid-encapsulated forms are less toxic than the traditional deoxycholate form, although they are much higher in cost.

Antifungal resistance in dogs and cats

The misuse of antifungal agents causes the development of resistance in some fungal species; however, it is known that some species are primarily resistant to certain drugs (Yegenoğlu, 2012). Therefore, several factors should be considered to successfully treat fungal diseases, such as accurate diagnosis of mycotic diseases and understanding the modes of action and mechanisms of resistance of antifungal agents. Antifungal resistance can also emerge in different animal species, highlighting the need to investigate the epidemiology of antifungal resistance in animals (Ziołkowska et al., 2014).

Antifungal resistance can be categorized into three main categories, namely, primary or intrinsic, acquired, and clinical resistance. Inherent or primary resistance (intrinsic resistance) describes isolates that possess an innate resistance to antifungal drugs. Resistance of *Candida krusei* to FCZ is an example of intrinsic resistance (Loeffler and Stevens, 2003). However, acquired resistance described isolates that are initially susceptible but develop secondary resistance mechanisms after exposure to antifungals.

Acquired antifungal resistance can be developed in animals due to the frequent and prophylactic use of antifungal agents (Loeffler and Stevens, 2003; Yegenoğlu, 2012). Clinical resistance is defined as the inability to eliminate the fungal agents (treatment failure) despite applying the necessary antifungal treatment procedures. The clinical failure of antifungal therapy could be attributed to multifactorial including, but not limited to, i) strain related factors such as the serotype of the fungal agent, ii) drug-related factors such as the dose, structure of the drug, and pharmacokinetics, and iii) host-related factors such as the state of the immune system, and the severity and the site of infection (Loeffler and Stevens, 2003; Bhanderi et al., 2009; Wiederhold, 2017; Geddes-McAlister and Shapiro, 2019).

Resistant *A. fumigatus* was developed due to sublethal concentrations of antifungal drugs (Bordallo-Cardona et al., 2017; Zhang et al., 2017). This explains the isolation of multi-resistant *A. fumigatus* from environmental samples such as compost heaps containing azoles residues used for crop protection (Snelders et al., 2012; Zhang et al., 2017). Accordingly, accurate diagnosis and optimum dose of antifungal drugs should be considered in treating fungal diseases in small animals.

Antifungal drugs used in small animals

Despite the recent pharmacological advances, therapeutic options against fungal infections are limited, and there are few licensed products for animals (Seyedmousavi et al., 2018). However, there are several classes of antifungal drugs that can be used in animal and human medicine, namely: i) azoles that target ergosterol biosynthesis, ii) echinocandins that inhibit fungal cell wall biosynthesis, iii) polyenes that bind to ergosterol in the fungal cell membrane, leading to cell lysis, and iv) pyrimidines, a nucleoside analog that disrupts the pyrimidine metabolism in the nucleus of the fungal cell, v) allylamines inhibit ergosterol biosynthesis (Foy and Trepanier, 2010; Perfect, 2017; Robbins et al., 2017; Seyedmousavi et al., 2018). For superficial mycoses, especially infections caused by dermatophytes, azoles, allylamines are the most effective drugs (Moriello, 2020). The main antifungal classes used in small animals and their mechanisms of action are shown in Table 1.

Antifungal drugs and development of resistance

Polyenes

Polyenes are the first antifungal agents developed against life-threatening mycoses through the fermentation of *Streptomyces nodosus*. Am-B, nystatin (NIS), and natamycin are included in this group. Am-B is highly effective against systemic fungal infections such as candidiasis, aspergillosis, blastomycosis, and coccidioidomycosis (Seyedmousavi et al., 2018).

Two mechanisms of action are described for polyenes: i) interaction with sterols in the fungal cell membrane; Am-B impairs membrane functions by affecting the permeability of the cell membrane. By binding to ergosterols, a unique cell membrane component, it creates pores in the fungal cell wall and increases the cell permeability, which in turn leads to

Table 1: Classification of antifungal agents, their target, mechanisms of action, and mechanisms of resistance development (Bhanderi et al., 2009; Yegenoğlu, 2012; Moriello, 2020).

Antifungal drug	Antifungal agent	Target and impact mechanisms	Mechanisms of resistance development
Polyenes	• Amphotericin-B • Nystatin	• Binding to ergosterol, ergosterol reduction • Oxidative damage to the fungal cell membrane	• Alterations in the sterol content of the plasma membrane • Resistance to oxidation • Alteration of cell wall
Azoles	• Ketoconazole • Miconazol • Fluconazole • Itraconazole • Voriconazole • Posaconazole	• Inhibition of cytochrome p450 14α-lanosterol demethylase (ERGII) sterol content change (ERG5)	• Over-expression of membrane transporters • Altered ergosterol biosynthesis • Altered sterol import • Chromosomal alteration
Pyrimidines	• 5-Flucytosine (5-FC)	• Inhibition of protein synthesis • Inhibition of nucleic acid synthesis	• Decrease drug uptake by alterations of permease • Limitation of the conversion of 5-flucytosine to 5-FU
Echinocandins	• Caspofungin • Anidulafungin • Micafungin • Aminocandin	• 1,3-β-D glucan synthase inhibition	• Prevention of echinocandins binding affinities with glucan by induction of mutations in the hotspot-1 and hotspot-2 regions of the two subunits of 1,3-β-D-glucan synthase complex
Allylamines	• Terbinafine	• Ergosterol biosynthesis inhibition	• Mutations in the squalene epoxidase gene that cause amino acid changes in the enzyme necessary for the ergosterol synthesis pathway

leakage of monovalent ions such as K^+, Na^+, H^+, and Cl^-, and subsequently followed by fungal cell death (Bhanderi et al., 2009). Mammalian cells are less sensitive to this drug because they have cholesterol instead of ergosterol (Sykes and Greene, 2013). Therfore, Am-B is the drug of choice because of its fungicidal activity, rapid onset of action, and parenteral dose availability. ii) oxidative damage to the fungal cell membrane; Am-B induced reactive oxygen species (ROS) that results in membrane disruption and cell death through membrane lipid peroxidation.

Mechanism of resistance

Clinical and *in-vitro* acquired resistance in polyenes is rare (Vanden Bossche et al., 2003). It usually develops slowly and does not reach high levels even after long-term treatment. Navarro and coworkers found five Am-B resistant isolates (out of 43 strains) of the genus *Trichosporon* obtained from the oral mucous samples of mixed-breed stray dogs in 2020. The authors shed light on the possible resistance of this genus to Amp-B and the importance of antifungal susceptibility tests (Navarro et al., 2020).

Polyenes have several mechanisms for developing resistance: i) alterations in the sterol content of the

plasma membrane; mutation of the ERG3 gene that encodes C-5 sterol desaturase leads to altered ergosterol expression, leading to resistance to Am-B (Young et al., 2003). Resistance to Am-B was found to be related to the accumulation of sterol intermediates, 3-β-ergosta-7,22-dienol and 3-β-ergosta-8-dienol (Nolte et al., 1997). The amount of membrane sterols of the NIS-resistant mutant *M. pachydermatis* strain has been proven to be significantly reduced compared to the wild-type strain. *A. terreus* is resistant to Am-B *in-vitro*, but a continuous clinical improvement has been observed after treatment of some dogs with lipid complex Am-B (Renschler, 2017).

Using *in-vitro* animal model, resistant *A. terreus* strains were found to have the lowest ergosterol content (Walsh et al., 1990). ii) resistance to oxidation; Am-B resistant strains produce more intracellular and extracellular catalase, which has an antioxidant effect and can remove ROS (Anderson et al., 2009; Blum et al., 2013). iii) biofilm formation; it is proposed that the slow growth rate of *Candida* spp. by forming a biofilm could be a mechanism of resistance development (Baillie and Douglas, 1998). iv) alteration of the cell wall; low chitin content of cell membrane is associated with increased resistance to Am-B in *C. albicans* (Bahmed et al., 2002, 2003).

Azoles

Azoles are widely used antifungal agents due to their low side effects and high antifungal activity. They are classified as imidazoles (KTZ, MCZ, clotrimazole (CLT)) and triazoles (FCZ, ITZ) according to the presence of two or three nitrogens in the azole ring (OR et al., 2000). Newly developed and under development new generation azoles (voriconazole, posaconazole, ravuconazole, albaconazole and isavuconazole) are in the triazole group (Kablan et al., 2009; Sargin et al., 2014). Azole compounds exert antifungal effects by inhibiting cytochrome P-450-dependent lanosterol 14α-demethylase, an enzyme involved in the synthesis of ergosterol (OR et al., 2000; Masiá Canuto and Gutiérrez Rodero, 2002).

Mechanism of resistance in azoles

Various studies have reported high levels of azole resistance (Seyedmousavi et al., 2018). Several mechanisms of resistance development were described: i) over-expression of membrane transporters (Adenosine Triphosphate-binding cassette transporters (ABC-T) and major facilitator transporters (MFS-T), causing hyper-susceptibility to azoles (Marger and Saier, 1993; Michaelis and Berkower, 1995; Cannon et al., 2009). ii) altered ergosterol biosynthesis through mutations or overexpression of ergosterol pathway genes namely ERG11/CYP51, ERG3, ERG6 (Bhattacharya et al., 2020). iii) altered sterol import in *C. albicans* by importing cholesterol and serum from the blood (Zavrel et al., 2013). The amount of membrane sterols of the NIS-resistant mutant *M. pachydermatis* reference strain has been significantly reduced compared to the wild-type strain. *A. terreus* is resistant to Am-B *in-vitro*, but a continuous clinical improvement has been observed after treatment of some dogs with lipid complex Am-B (Renschler, 2017). Since sterols are the main target of NIS-containing polyene antifungal agents, it has been acknowledged that this reduction is directly related to the increased MICs observed for resistant mutants (Peano et al., 2020). iv) chromosomal alteration, such as loss of heterozygosity and aneuploidy, is reported in *C. albicans* (Ford et al., 2014) and *C. glabrata* (Poláková et al., 2009).

Azole resistance in Candida species

Azole resistance in human isolates has been known since the onset of Acquired Immune Deficiency Syndrome (AIDS) (Arendrup and Patterson, 2017). However, the investigation of the antifungal susceptibility of *Candida* spp in animals is very new. Molecular mechanisms of azole resistance in *Candida* spp. include the reduced affinity of the target for azole, e.g., lanosterol demethylase, an energy dependent efflux pump mechanism that leads to the decrease in the intracellular accumulation of azoles, upregulation of the target enzyme as a result of suppression of the antifungal agent, and the development of bypass pathways where ergosterol is replaced by its precursor 14α-methyl-fecosterol (Peano et al., 2020).

Changes in the target enzyme due to point mutations in the ERG11 coding gene result in decreased susceptibility to azoles. Overexpression of the CDR1, CDR2, and MDR1 genes encoding efflux pumps lead to azole resistance (Seyedmousavi et al., 2018). All the resistance mechanisms mentioned are effective on the decreased susceptibility to azoles in *C. albicans*. A few of these mechanisms are also found in other *Candida* spp. known to be associated with resistance to azoles. Regarding *C. neoformans*, resistance has been shown to result from upregulation or modification of the target enzyme, reduced drug availability to the target, or combinations of these mechanisms (Castelo-Branco et al., 2020).

Candida spp. was detected in the study made from samples taken from 13 dogs and seven cats with urinary tract infections. The *C. albicans* isolate was initially susceptible to FCZ, ITZ, and KTZ; however, it became resistant after intermittent oral FCZ therapy for two months. It has been reported that a *C. glabrata* isolate is resistant to KTZ, ITZ, and FCZ (Pressler et al., 2003). In a case report, *C. glabrata* was isolated from the urine sample collected from a dog with urinary tract infection, diabetes ketoacidosis, and acute pancreatitis and was resistant to azole antifungals. In their analysis to understand the azole resistance mechanism in this isolate, the authors revealed that increased drug efflux is mediated by overexpression of the ATP transporter genes CDR1 and PDH1 (Kim et al., 2017).

In addition, weak acids used in industrial dog foods for preservation affect the resistance mechanism. Deteriorated food yeasts develop flow pump-mediated resistance to weak acids (Ullah et al., 2012). Furthermore, using pesticides and anthelmintic agents in dogs

contributes to the development of azole resistance by increasing P-glycoproteins and efflux pump activity (Castelo-Branco et al., 2020).

Azole resistance in Aspergillus spp.

Triazole antifungal agents are used to prevent and treat infections caused by *Aspergillus* spp. Azole resistance in *A. fumigatus* results from point mutations in the CYP51A gene, which encodes the enzyme responsible for converting lanosterol to ergosterol (Seyedmousavi et al., 2015). It has been reported that the isolate from a sick dog has multiazole resistance, and there is a mutation associated with azole resistance (F46Y) in the Cyp51A gene sequencing (Talbot et al., 2015). With the widespread use of azoles in medical applications and agriculture, the emergence of triazole resistance in *A. fumigatus* caused by mutations in the Cyp51A gene is a global public health problem (Seyedmousavi et al., 2018).

Brachiocephalic cat breeds, especially Persian and Himalayan cats, are prone to upper respiratory tract aspergillosis (Dokuzeylul et al., 2013; Barrs and Talbot, 2014). Resistance to FCZ and some triazole drugs have been detected in aspergillosis infections in cats. (Barachetti et al., 2009; Barrs and Talbot, 2014). In a study to detect azole resistance in *A. fumigatus* isolates in sino-nasal aspergillosis cases in cats and dogs, most of the collected isolates had high MIC values for KTZ and low for CLT and enilconazole. In contrast, ITZ, posaconazole, and voriconazole were lower than the established epidemiological cut-off values (Talbot et al., 2015).

Azole resistance in Malassezia spp.

In dogs, many studies and case reports documented azole resistance and treatment failure of *Malassezia* spp. Studies show that the defense mechanisms of *M. pachydermatis* against azoles may depend on efflux pumps, which is a common azole resistance mechanism in *Candida* species (Iatta et al., 2016). The development of antifungal resistance against *Malassezia* spp. in canines is rare. In a prospective study conducted in Australia, treatment failure based on resistance to antifungal agents was detected in seven cases in which *Malassezia* yeasts were detected by cytological methods (Robson et al., 2010).

In Japan, a resistant isolate with mutations in the ERG11 gene encoding lanosterol 14α-demethylase, the target site of antifungal azoles, was reported (Kano et al., 2019). Another recently evaluated possibility is the chromosomal rearrangement and gene overexpression, as common resistance mechanisms in other fungal species. Kim and coworkers found that one region on chromosome 4 of two isolates of *M. pachydermatis* quadrupled in tandem. It increased the expression of genes located in the region, including ERG11 and ERG4 targeted by KTZ (Kim et al., 2018). In a case reported from Italy, they found that *M. pachydermatitis* strains from a Toy Poodle dog that did not respond to azole treatment had several times higher MIC values than strains from untreated dogs. Azole agents (Posaconazole, FCZ, KTZ), which were not used in this case, showed decreased activity *in-vitro*, revealing a possible cross-resistance of different azoles in *M. pachydermatis* (Angileri et al., 2019).

However, given that a standard procedure for susceptibility testing of *M. pachydermatis* still does not exist, resistant cases may have been underreported in the literature due to the difficulty of obtaining laboratory approval. It was reported that an Am-B treatment successfully treated a kitten with *C. neoformans* after treatment failed with FCZ. The clinical course of the kitten presented in this case report may indicate resistance to azoles. The molecular mechanisms behind azole resistance in *C. neoformans* have been demonstrated by ERG11 expression and increased efflux pumps (Vercelli et al., 2021).

Pyrimidine

Flucytosine is the only systemic antifungal agent belonging to the class of nucleoside analogs. Flucytosine is converted to 5-fluorouracil by cytosine deaminase in the fungal cell and alters protein and DNA synthesis. The activity of flucytosine is limited to *C. neoformans* and yeasts such as *Candida* spp. It is used in combination with Am-B to treat systemic cryptococcosis in cats. However, this combination is not recommended in dogs because of the potential for severe toxic reactions (Seyedmousavi et al., 2018). There are two mechanisms of action of flucytosine: i) inhibition of amino acid synthesis by conversion of 5-fluorouracil through 5-fluorouridine monophosphate (FUMP) and 5-fluorouridine diphosphate (FUDP) to 5-fluorouridine triphosphate (FUTP). The FUTP alters the aminoacylation of tRNA and prevents amino acid synthesis. ii) inhibition of nucleic acid synthesis by inhibition of thymidylate synthase (Mayers et al., 2017).

Mechanism of resistance

Two main mechanisms of pyrimidines resistance have been described: i) decreased drug uptake by alterations of permease, encoded by FCY2 gene, activity. This mechanism was reported as primary resistance *S. cerevisiae* and *C. glabrate*. ii) limitation of the conversion of 5-flucytosine to 5-FU, secondary resistance. This mechanism is achieved by alteration of cytosine deaminase (encoded by FCY1) or uracil phosphoribosyltransferase (encoded by FUR1 genes) activities (Jund and Lacroute, 1970; Whelan, 1987; Ghannoum and Rice, 1999; Espinel-Ingroff, 2008). There are many case reports of natural and acquired resistance of flucytosine to *Cryptococcus* spp. (Bermas and Geddes-McAlister, 2020). Resistance to flucytosine can develop rapidly, even during treatment; therefore, it is not recommended alone to treat mycotic infections. *Rhodotarula mucilaginosa* and *Trichospora jiirovecii* were detected in a mycological culture sample of a dog with broncho-tracheitis. Both isolates were reported to be resistant to flucytosine, Am-B, and MCZ in the antifungal susceptibility test (Biegańska et al., 2018). Also, *Aspergillus deflectus* and *Neosartoria fischeri* isolated from two German shepherds were resistant to Am-B, flucytosine, FCZ, and ICZ (Krockenberger et al., 2011).

Echinocandins

Echinocandins are the only class of antifungal agents that directly target the fungal cell wall. Caspofungin, micafungin, and anidulafungin obtained from *Aspergillus rugulovalvus* are members of this group. Echinocandins inhibit cell wall synthesis by inhibiting the β-1,3-D-glucan synthase that catalyzes the biosynthesis of β-1,3-D-glucan, a crucial fungal cell wall component. Mammalian cells do not contain this enzyme, and therefore direct human cell toxicity is minimal (Perlin et al., 2017).

Mechanism of resistance in echinocandins

The development of echinocandin resistance in susceptible species is always acquired through mutations in the hotspot-1 and hotspot-2 regions of the two subunits of 1,3-β-D-glucan synthase complex, encoded by FKS1 and FKS2 genes (Pfaller, 2012; Sanguinetti et al., 2015; Kołaczkowska and Kołaczkowski, 2016). Detection of the echinocandin resistance could be predicted by sequence analysis of FKS genes, the most accurate method to predict treatment failure (Lewis et al., 2013; Khan et al., 2018). Al-Baqsami and others found that all micafungin-resistant isolates harbored a nonsynonymous/deletion mutation in hotspot-1 of FKS2 (Al-Baqsami et al., 2020). In a study to detect yeast species found in different anatomical regions of healthy dogs, *Malassezia pachydermatis* was the most isolated fungi, and it was found to be sensitive to ITZ, FCZ, KTZ, and Am-B but resistant to caspofungin (Brito et al., 2009). Although echinocandins show promise in veterinary medicine for treating systemic yeast infections, their potential to treat generalized dimorphic fungal infections in dogs and cats is weak (Seyedmousavi et al., 2018).

Allylamines

Allylamines include terbinafine (Lamisil), flunarizine, and naftifine. It is a primary fungicide effective against dermatophyte infections (Sanglard et al., 2009). Terbinafine is readily absorbed from the gastrointestinal tract and rapidly diffuses from the bloodstream into all skin tissue, including the dermis and epidermis. Concomitant use of terbinafine with other antifungal drugs in various fungal infections may increase drug effectiveness. However, terbinafine in dogs and cats is limited to treating *Malassezia* and *Dermatophytes* infections (Kano et al., 2019; Schlemmer et al., 2019; Peano et al., 2020). Allylamines block the conversion of squalene to lanosterol, resulting in an accumulation of squalene in the membrane and a decrease in ergosterol (Ozkan et al., 2003; Seyedmousavi et al., 2018).

Mechanism of resistance

Resistance to terbinafine and other allylamines is rare and is usually associated with point mutations in the squalene epoxidase gene that causes amino acid changes in the enzyme necessary for the ergosterol synthesis pathway (Łagowski et al., 2020). Martins and coworkers in 2016, reported that resistance of *Dermatophytes* to antifungal drugs is often due to overexpression of genes encoding ATP-binding cassette (ABC) transporter proteins, including pleiotropic drug resistance (PDR1) and multidrug resistance (MDR2 and MDR4) genes (Martins et al., 2016). In 2018, the first terbinafine-resistant *M. canis* strain was reported in a cat (Hsiao et al., 2018). However, the terbinafine resistance mechanism of the *M. canis* strain has not been fully defined. The mechanism is thought to be formed due to the overexpression of genes that encode ABC transporter proteins (Kano et al., 2018). Terbinafine resistance was also reported in *Trichophyton rubrum* isolated from a human dermatophytosis patient (Digby et al., 2017). Nevertheless, Łagowski et al. (2020), stated that drug exposure is not required to develop resistance to terbinafine, and the resistance can develop intrinsically, as proved by identifying terbinafine-resistant *T. mentamentagrophytes* strains isolated from asymptomatic foxes.

Conclusions

Animals can spread antifungal-resistant isolates to humans and contaminate the environment. Several circumstances such as suboptimal length of treatment, incorrect diagnosis, the pharmacokinetics of drugs, immune status of the animals, the severity, and the site of infection are the main factors for the emergence of antifungal resistance. Since small animals could also be a source for the emergence of antifungal resistance, understanding the One Health concept to control the emergence of fungal resistance is urgently needed. Meanwhile, further research is required to fully understand the mechanisms involved in the emergence of antifungal resistance in small animals.

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