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Review paper

A comprehensive review of the taxonomy, pathogenesis, and therapeutic challenges of *Malassezia pachydermatis* in companion animals

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Abstract

Malassezia pachydermatis is a lipophilic yeast that forms part of the skin microbiota of dogs and cats, and can transform into an opportunistic pathogen when the microbial equilibrium is disrupted. It is most commonly implicated in cases of otitis externa and dermatitis in dogs, and is increasingly isolated from cats as well, particularly in instances of otitis externa. The rising number of infections reported in recent years underscores the growing significance of this microorganism in veterinary medicine. Despite its clinical relevance, standardized methods for assessing the antifungal susceptibility of *M. pachydermatis* are still lacking. Existing studies vary in the culture used, incubation conditions, and interpretation of minimum inhibitory concentration (MIC) values, which complicates the comparison of results and the evaluation of the true extent of antifungal resistance. Of particular concern is the increasing resistance to azoles, which remain the cornerstone of therapy for *Malassezia*-associated infections. Due to the limitations in efficacy and safety of conventional antifungal drugs, natural compounds with antifungal activity, including essential oils, are receiving increasing attention. These oils are mixtures of volatile plant metabolites with a broad spectrum of biological activity, including antibacterial, anti-inflammatory, and antifungal properties. Selected essential oils, such as oregano and thyme oils, have been shown to exhibit significant activity against yeasts of the genus *Malassezia*. Their potential therapeutic application represents a promising research direction, particularly in the context of increasing resistance to conventional antifungal agents.

Keywords: *Malassezia pachydermatis*, dogs, *Malassezia* infections, essential oils

Introduction

The genus *Malassezia* (*M*.), comprising lipophilic yeasts that are commensals of the skin of humans and animals, plays a key role in veterinary dermatology. Among its members, *Malassezia pachydermatis* holds a unique position as the only species that does not exhibit a strict requirement for exogenous lipids. This microorganism constitutes a permanent, typically non-invasive component of the skin and mucosal microbiome of healthy dogs and, less frequently, cats. (Guillot and Bond 1999) However, under conditions of disrupted skin homeostasis – such as allergic diseases, disorders of keratinization or immunodeficiencies – this otherwise harmless commensal may become an opportunistic pathogen. *M. pachydermatis* is one of the main etiological agents of dermatitis and otitis externa in dogs, and it is increasingly being isolated also in cases of dermatoses in cats.

Over the past decades, the clinical importance of *M. pachydermatis* has increased considerably, particularly in veterinary dermatology, where it is recognized as one of the primary etiological agents of recurrent and chronic skin infections in companion animals. Despite this, significant challenges remain in both diagnostics and treatment. In particular, the growing number of reports describing reduced susceptibility to commonly applied antifungal agents, especially azoles, raises concerns regarding therapeutic efficacy and long-term disease management. (Bond et al. 2020)

Several review articles have addressed various aspects of *Malassezia* biology, ecology, and clinical relevance (Bond et al. 2020, Peano et al. 2020, Bajwa 2023). However, these studies predominantly focus on general characteristics of the genus or on clinical and dermatological manifestations, while key emerging issues remain insufficiently explored. More recently, Hobi et al. (2022) reviewed the zoonotic implications and cross-species differences in *Malassezia* colonization, yet this aspect remains only marginally addressed in the veterinary literature (Hobi et al. 2022). Notably, there is a lack of integrative analyses that combine current knowledge on molecular mechanisms of antifungal resistance with methodological limitations in susceptibility testing and their clinical implications. Furthermore, although alternative therapeutic approaches particularly the use of natural compounds such as essential oils are increasingly investigated, their potential role in the management of *M. pachydermatis* infections has not been comprehensively synthesized within a broader resistance-focused framework.

Another major limitation in the field is the absence of standardized protocols for antifungal susceptibility testing of *M. pachydermatis*. Variability in culture media, incubation conditions, and interpretation of minimum inhibitory concentration (MIC) values across studies significantly hampers data comparability and prevents the establishment of clinically relevant breakpoints. This gap not only complicates the assessment of resistance rate but also limits the development of evidence-based therapeutic guidelines. (Cafarchia et al. 2012, Weiler et al. 2013, Álvarez-Pérez et al. 2016, Nojo et al. 2025)

Beyond its clinical relevance in companion animals, *M. pachydermatis* also possesses a recognized, though still underappreciated, zoonotic potential. Direct contact with colonized dogs and cats has been associated with cutaneous carriage in human caregivers, and sporadic cases of invasive infection – including catheter-related fungemia – have been reported in humans, particularly in neonates and children receiving lipid-containing parenteral nutrition (Hobi et al. 2022, Teoh et al. 2022). Despite these findings, the public health implications of *M. pachydermatis* as a potential zoonotic agent remain insufficiently addressed in the existing veterinary literature.

In this context, there is a clear need for a critical and integrative review that not only summarizes existing knowledge but also identifies current gaps and future research directions.

Therefore, the aim of this review is to provide a comprehensive and critical synthesis of current knowledge on *M. pachydermatis*, with particular emphasis on aspects that remain underrepresented in previous reviews. Specifically, this article focuses on: molecular mechanisms underlying antifungal resistance, challenges associated with the lack of standardized susceptibility testing methods, and the potential of natural compounds, including essential oils, as alternative or adjunctive therapeutic strategies. By integrating these perspectives, this review aims to provide a more coherent framework for understanding the therapeutic challenges associated with *M. pachydermatis* infections and to support the development of more effective and targeted treatment approaches.

Taxonomy

Multigene phylogenetic analyses have demonstrated that the genus *Malassezia* has deep evolutionary roots within the Ustilaginomycotina and belongs to the distinct class Malasseziomycetes. (Wang et al. 2014, Wu et al. 2015). Initially, the genus was thought to comprise only two species: the lipid-dependent *M. furfur* and the lipophilic *M. pachydermatis*. However, further molecular studies enabled the identification of numerous new species, using LSU rDNA and ITS sequences, as well as additional genetic markers such as CHS2,

TEF1, and beta-tubulin. (Boekhout et al. 2010, Castellá et al. 2014). During studies based on the analysis of 164 eukaryotic genes from fourteen *Malassezia* species, three major groups within the genus were distinguished: Cluster A, comprising species most frequently found on human skin, such as *M. furfur*, *M. japonica*, *M. obtusa*, and *M. yamatoensis*; Subcluster B1, including the most commonly encountered *Malassezia* species on human skin, namely *M. globosa* and *M. restricta*; Subcluster B2, comprising *M. sympodialis*, *M. dermatis*, *M. caprae*, *M. equina*, *M. nana* and *M. pachydermatis*; and Cluster C, representing the most ancestral evolutionary lineage, represented by *M. cuniculi* and *M. slooffiae*. (Wu et al. 2015). However, in subsequent years, three new species were discovered and described: *M. brasiliensis* and *M. psittaci* (isolated from parrots), and *M. arunalokei* (isolated from human skin) (Cabañes et al. 2016, Honnavar et al. 2016, Theelen et al. 2018). Genetic studies have demonstrated that *M. pachydermatis* exhibits high intraspecific variability, which may indicate adaptation to different host species. Analyses of LSU rRNA sequences, restriction fragment length polymorphism (RFLP), and enzymatic assays revealed associations between specific fungal subtypes and the animal species from which the isolates were obtained. (Guillot et al. 1997, Guillot and Bond 1999).

Morphological and physiological characteristics

Colonies of *M. pachydermatis* grown on Sabouraud agar at 32°C are convex, dull, and pasty, with colors ranging from white to light brown, and reach a diameter of 3-5 mm after 7 days (Guillot and Bond 1999). The cells are usually oval or ellipsoidal in shape (2-6 × 2-7 µm), with small-cell and large-cell types distinguished, as well as spherical and rod-shaped forms. (Kiss et al. 1996, Guillot and Bond 1999). Electron microscopy revealed a thick, multilayered cell wall with a characteristic undulating inner surface. Reproduction occurs by unipolar budding. Under microaerophilic or stress conditions, elongated or hyphal-like cells have occasionally been observed. However, *M. pachydermatis* does not form true hyphae or pseudohyphae, and these structures are considered aberrant morphological forms rather than genuine filamentation. (Faergemann and Bernander 1981, Guillot and Bond 1999).

M. pachydermatis is able to grow without the addition of long-chain fatty acids. (Guillot and Bond 1999, Wu et al. 2015), and is the only species within the genus *Malassezia* that can grow under laboratory conditions without the need for lipid supplementation of the culture medium (Guillot and Bond 1999). In contrast to other *Malassezia* species, which require the presence of additional fatty acids, individual *M. pachydermatis* strains may differ in their growth rate and lipid dependence; however, this dependence usually disappears after several passages. The species is capable of utilizing mannitol, glycerol, and sorbitol as carbon sources, hydrolyzing urea, and using both organic and inorganic nitrogen sources (Guillot and Bond 1999, Hossain et al. 2007). The lack of sugar fermentation ability confirms its affiliation with the phylum *Basidiomycota*. *M. pachydermatis* strains exhibit enzymatic activity of phosphatases, esterases, lipases, peroxidases, and lecithinase, whereas they do not show the ability to coagulate or agglutinate (Kiss et al. 1996). Pathogenic isolates from diseased skin exhibit higher phospholipase production, and the enzyme activity correlates with genetic subtypes (Cafarchia et al. 2008). The yeasts are capable of degrading proteins in media containing serum or casein and exhibit resistance to cycloheximide, whereas some strains are sensitive to lauric acid (Ushijima et al. 1981, Guillot and Bond 1999).

M. pachydermatis grows over a wide temperature range (25-41°C), with an optimum at 37°C, and grows well under aerobic and microaerophilic conditions, whereas growth under anaerobic conditions is limited. In addition, *M. pachydermatis* grows well under capnophilic conditions. Cells can be preserved by lyophilization, or storage in liquid nitrogen (Guillot and Bond 1999). The lipid profile of the cells shows a predominance of unsaturated fatty acids over saturated ones and varies depending on the strain. The coenzyme Q9 system and membrane sterols, including ergosterol, influence susceptibility to polyene antibiotics (Breierová et al. 1991, Uchida et al. 1994).

Skin colonization: distribution and predisposing factor

Yeasts of the genus *Malassezia* are permanent commensals of the skin of many mammals and birds, whereas their occurrence in the natural environment is limited. Among them, *M. pachydermatis* colonizes the skin of wild and domestic carnivorous animals, such as dogs, cats, ferrets, foxes, and bears. This species has also been detected in other animals, including rhinoceroses, pigs, horses, birds, and marine mammals (pinnipeds), while its presence has not been confirmed in rodents and lagomorphs. Some species, such as pigs, cats, and rhinoceroses, may be simultaneously colonized by *M. pachydermatis* and other lipid-dependent species of this genus (Guillot and Bond 1999).

Colonisation has been particularly well studied in dogs and cats, reflecting the clinical importance

of *M. pachydermatis* in these animals. In dogs, colonization likely begins within the first days of life, with yeasts transmitted from the maternal flora during licking and nursing (Bond et al. 2020). An analysis of the distribution of *M. pachydermatis* in adult, healthy dogs of different breeds showed that the areas most frequently colonized were the muzzle and lips (81%) and the interdigital skin (60%) (Kennis et al. 1996). Yeasts were less frequently detected in the axillary region (12.5%), the inguinal area (23%), and on the dorsum (4%). A relatively common habitat was the perianal skin and anal mucosa (55%), whereas the oral cavity and nasal cavity were rarely colonized. The distribution and abundance of yeasts may depend on breed; in basset hounds, a higher frequency of colonization and greater yeast densities were observed in the nose, muzzle, vulva, and groins (Bond and Lloyd 1997). In cats, although *M. pachydermatis* is the dominant species, it is isolated less frequently than in dogs. The population density of these yeasts varies considerably depending on body site and breed. Commensal populations constitute a reservoir that, in the presence of host predisposing factors, may lead to excessive proliferation and inflammatory responses (Bond et al. 2020).

Recent epidemiological data confirm the continued clinical prominence of *M. pachydermatis* in companion animal populations. A five-year retrospective analysis of canine clinical samples identified *M. pachydermatis* as the predominant yeast isolate associated with otitis and dermatitis in dogs (Dinkova and Rusenova 2024). In cats, comparative studies of commensal yeast species have further shown that *M. pachydermatis* differs from co-occurring yeasts, such as those isolated from facial hair, in its antifungal susceptibility profile, highlighting the value of species-level identification even among commensal isolates (Yurayart et al. 2024).

Excessive proliferation of *M. pachydermatis* is promoted by diseases that increase moisture, alter lipid composition, or disrupt epidermal barrier function, as well as by abnormal immune responses (Bond et al. 2020). Pruritic diseases, such as atopic dermatitis, modify the skin microenvironment by increasing moisture (salivation), damaging the barrier (scratching), and enhancing sebum production. Although not all studies confirm a strong correlation between atopic dermatitis and the proliferation of *M. pachydermatis*, excessive growth of *Malassezia* spp. has been observed in both dogs and cats with allergic dermatoses (Plant et al. 1992). Another important factor is seborrheic disease (primary and secondary), during which significantly higher numbers of *M. pachydermatis* are reported on the skin of dogs (Bond and Lloyd 1997) and cats (Åhman et al. 2007) compared with healthy animals. Disruption of the local microbial balance by antibacterial treatment may also predispose to yeast overgrowth: a recent study demonstrated that successful topical antibacterial monotherapy of canine bacterial otitis externa was frequently followed by secondary *Malassezia* overgrowth, likely reflecting the loss of competitive inhibition normally exerted by the bacterial microbiota (Juhola et al. 2025). Disorders associated with impaired epidermal barrier function due to keratinization abnormalities, such as zinc-responsive dermatosis and hepatocutaneous syndrome, also predispose to yeast overgrowth (Bond et al. 2020). In cats, *Malassezia* overgrowth is also observed in thymoma-associated dermatitis and paraneoplastic alopecia (Mauldin et al. 2002).

Although age and sex are not significant factors in dogs in the context of *Malassezia*, infections and colonisations, breed predisposition is well documented. Breeds that are particularly susceptible include the West Highland White Terrier, English Setter, Basset Hound, American Cocker Spaniel, Australian Silky Terrier, Dachshund, Shih Tzu, Poodle, and Boxer. A common feature of these breeds is a tendency to form skin folds, which increases the risk of infection (Scott and Miller 1989, Mauldin et al. 2002). In cats, a predisposition to excessive proliferation of *M. pachydermatis* has been observed in the Devon Rex and Sphynx breeds, with the highest yeast densities found in skin folds around the claws (Colombo et al. 2007, Åhman and Bergström 2009).

Pathogenicity and host response

The pathogenicity of *M. pachydermatis* is opportunistic in nature and results from the combined effects of yeast virulence factor production and an abnormal or excessive host immune response. The development of symptomatic infection occurs when the balance between these two components is disrupted. According to the accepted hypothesis, *M. pachydermatis* residing on the skin surface produces antigens that may penetrate into the deeper layers of the epidermis. There, they are captured by antigen-presenting cells (APCs), including Langerhans cells located in the epidermal layers and dendritic cells present in the dermis, which may initiate the host immune response. These cells migrate to the regional lymph nodes, where they present antigens to T lymphocytes via major histocompatibility complex class II (MHC II) molecules. In the presence of specific cytokines, Th0 lymphocytes become activated and differentiate into Th1 or Th2 subpopulations. Predominance of IL-12 promotes the development of a Th1 response, whereas dominance of IL-4 and IL-13 leads to a Th2 response. Th lymphocytes activate B lymphocytes, stimulating their differentiation

into plasma cells that produce antibodies. Th1 cells support IgG production, whereas cytokines secreted by Th2 cells induce immunoglobulin class switching to IgE. Although Th1 and Th2 responses are often described as distinct pathways, both mechanisms may occur simultaneously during the immune response to *Malassezia*.

Cell-mediated immunity plays a central role in controlling *Malassezia* proliferation, and impairment of T-cell function may promote excessive yeast growth (Chen and Hill 2005). Studies evaluating peripheral blood mononuclear cells (PBMCs) demonstrated significantly stronger proliferative responses to *M. pachydermatis* in dogs with atopic dermatitis and yeast overgrowth than in healthy dogs (Mauldin et al. 2002). Yeast extracts stimulated PBMC proliferation in both healthy and atopic dogs, suggesting that *M. pachydermatis* may produce mitogens or superantigens (Chen and Hill 2005).

The humoral immune response also contributes to disease pathogenesis. Dogs with *M. pachydermatis*-associated dermatitis exhibit significantly higher serum levels of *Malassezia*-specific IgG and IgA than healthy animals (Bond et al. 1998). Similarly, dogs with atopic dermatitis have elevated levels of *Malassezia*-specific IgG regardless of the presence of yeast overgrowth (Nuttall and Halliwell 2001). *Malassezia*-specific IgG antibodies may play a protective role but may also initiate inflammatory reactions through complement activation. In contrast, IgE antibodies play a central role in type I hypersensitivity. Following sensitization, *Malassezia* allergens cross-link IgE molecules bound to the surface of mast cells, triggering degranulation, release of inflammatory mediators, and the development of immediate hypersensitivity reactions (Chen and Hill 2005). The importance of IgE-mediated mechanisms has been confirmed by intradermal testing. Dogs with atopic dermatitis, particularly those with confirmed *Malassezia* overgrowth, develop immediate hypersensitivity reactions following exposure to *M. pachydermatis* extracts, whereas healthy dogs do not. Such reactions are uncommon in non-atopic dogs with *Malassezia*-associated dermatitis. In addition to mediating mast cell activation, IgE antibodies may bind to Langerhans cells, enhancing allergen uptake and presentation during subsequent exposure (Chen and Hill 2005).

Although the mechanisms of *Malassezia* antigen penetration in canine skin have not yet been directly demonstrated, experimental studies have shown that topical application of an *M. pachydermatis* suspension to healthy canine skin induces lesions resembling those observed in naturally occurring infection, suggesting that the yeast or its antigens are capable of penetrating the skin and exerting pathogenic effects (Bond et al. 2004). Indirect evidence for transepidermal antigen penetration comes from dogs with atopic dermatitis, in which increased numbers of clustered Langerhans cells have been observed within skin lesions (Olivry et al. 1996). These cells also express surface-bound IgE, further supporting their role in allergen uptake and antigen presentation (Olivry and Hill 2001).

Diagnosis of Malassezia infections in dogs and cats

The diagnosis of *Malassezia* infections begins with a clinical examination performed by a veterinarian, who assesses the location of skin lesions, clinical signs, and orders appropriate diagnostic tests (Bajwa 2017). Lesions caused by *Malassezia* spp. may be localized or generalized and most commonly involve skin folds, axillae, inguinal areas, interdigital spaces, ear canals, the abdomen, the vulvar and perianal regions, as well as the lip margins, neck, face, or tail. In some cases, paronychia with characteristic nail discoloration is observed (Bond et al. 2020). Skin lesions are characterized by erythema, yellow, brown, or gray keratin – sebaceous scales, pruritus that may lead to self-induced alopecia, and, in chronic cases, lichenification and hyperpigmentation. In some breeds, such as West Highland White Terriers, chronic infections are often associated with hyperpigmentation, whereas in Basset Hounds it occurs less frequently (Bond et al. 2020). In cats, the clinical presentation often resembles seborrheic folliculitis, with initial involvement of the face and subsequent spread to the limbs and other body areas. Otitis externa manifests as head shaking, scratching, excessive discharge, and pain, and in chronic cases as erosions, ulcerations, and hyperplastic changes of the auricles (Guillot and Bond 1999). A rare but clinically important variant, suppurative *Malassezia otitis* externa, has also been described, presenting with dark brown mucoid discharge, canal ulceration, and cytological evidence of biofilm-like material, and requiring more aggressive management than typical *Malassezia otitis* (Nunes Rodrigues and Vandenabeele 2021, Nolitt and Drake 2025). Typical signs also include seborrhea in the form of scales, waxy or greasy discharge, crusts, papulo-crustous lesions, paronychia with discoloration of the nail bed, localized or generalized alopecia with erythema, and intertrigo in skin folds (Bajwa 2017).

After diagnosis, the next step is to assess yeast density. The most commonly used methods are cytological techniques (impression smears, adhesive tape preparations, scrapings) and culture-based methods, including the cup-scrub technique and contact plates (Bond et al. 2020). The cup-scrub method involves collecting

microorganisms from a defined area of skin using a cylinder (cup) filled with a buffered solution containing a detergent. In contrast, the contact plate method consists of applying special culture plates with raised agar directly to the examined surface, allowing microorganisms to be sampled directly from the skin. Dispersal techniques more accurately reflect actual skin colonization, and yeast density is assessed by counting cells in a defined number of microscopic fields. Comparative studies have shown that the cup-scrub method and contact plates yield strongly correlated results, whereas adhesive tape preparations are less sensitive at low yeast densities (Bond et al. 2020).

Modified Dixon's medium enables rapid colony growth and supports the development of lipid-dependent strains, whereas Sabouraud agar supplemented with 1% Tween 80 is less effective for fastidious isolates. Optimal incubation conditions are 32-37°C for dogs and 32-34°C for cats for at least 3-7 days (Bond et al. 2020). These methods are widely used in clinical diagnostics, evaluation of treatment efficacy, and studies of breeds predisposed to excessive *Malassezia* colonization (Bond and Lloyd 1997).

Histopathological changes in *Malassezia* infections include epidermal and follicular infundibular hyperplasia, hyperkeratosis, lymphocytic exocytosis, and the presence of yeasts in the stratum corneum (Mauldin et al. 2002). Edema and inflammatory infiltrates in the dermis are frequently observed, consisting mainly of lymphocytes, as well as plasma cells, histiocytes, neutrophils, and eosinophils. Histopathology is primarily useful as a complementary method for assessing inflammation and predisposing conditions, but it should not be used as the sole basis for diagnosis, particularly since a similar histological pattern may be seen in cases of canine atopic dermatitis (Mauldin et al. 1997, Bond et al. 2020).

Molecular methods, such as PCR, qPCR, NGS, and MALDI-TOF mass spectrometry, enable precise identification of *Malassezia* species, detection of resistant strains, analysis of the skin microbiome, whereas MLST and RAPD are used for genotyping (Kolecka et al. 2014). Sequencing of the 26S rRNA, ITS, CHS2, and ß-tubulin genes allows species and genotype typing, assessment of intraspecific variability, and evaluation of host adaptation (Guillot et al. 1997, Gupta et al. 2000, Puig et al. 2016). Molecular techniques complement traditional cytological and culture-based methods, enable analysis of samples without cultivation, detection of resistance-associated mutations, and personalization of therapy. Limitations include the high cost of equipment and reagents, the risk of contamination, and the need for qualified personnel (Bond et al. 2020).

The diagnosis of *Malassezia* infections should be based primarily on cytological and culture-based examinations. Histopathological and molecular methods play a complementary role, enabling detailed analysis of lesions and precise species typing. Integration of all approaches increases diagnostic accuracy and supports effective treatment and prevention (Bond et al. 2020).

Treatment strategies for infections caused by *Malassezia pachydermatis*

Treatment of fungal infections involves several main classes of drugs with different mechanisms of action, allowing therapy to be tailored to the specific fungal species, as well as the location and severity of the infection. Management of *M. pachydermatis*-induced dermatitis includes topical, systemic, or combination therapy, depending on the extent of lesions and the patient's overall health. For mild or localized lesions, frequent use of topical preparations containing antifungal agents such as ketoconazole, miconazole, climbazole, chlorhexidine, lime sulfur, enilconazole, or selenium sulfide is effective. Shampoos with dual active ingredients often demonstrate higher efficacy. Additionally, wipes or pads impregnated with chlorhexidine or climbazole can be used topically to effectively eliminate *M. pachydermatis*. In cases of more extensive involvement or multiple lesion sites, the most effective approach is combining topical therapy with systemic administration of antifungal drugs, such as ketoconazole, fluconazole, itraconazole, or terbinafine (Bajwa 2017).

Antifungal susceptibility testing is important for effective treatment and resistance management. Both reference and commercial methods (microdilution, agar-based tests) are used, standardized by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI). Reference methods are considered the gold standard but are time-consuming and demanding, which is why faster and less expensive commercial tests are often used in routine diagnostics. EUCAST and CLSI establish clinical breakpoints for minimal inhibitory concentrations (MICs) to determine whether a strain is susceptible or resistant. For *M. pachydermatis*, such breakpoints have not yet been established. Although studies evaluating the susceptibility of this species are available, owing to the specific growth requirements of *M. pachydermatis*, different methods (e.g., the use of various culture media) have been employed, preventing the standardization of results (Houšť et al. 2020).

According to Álvarez-Pérez et al. (2016), the MIC90 values for individual antifungal agents were as follows: AmB (amphotericin B) – 0.25 µg/mL, FCZ (fluconazole) – 32 µg/mL, KTZ (ketoconazole) – 0.5 µg/mL, ITZ (itraconazole) – 0.063 µg/mL, PSZ (posaconazole) – 0.5 µg/mL, TER (terbinafine) – 1 µg/mL, and VCZ (voriconazole) – 1 µg/mL. In the study by Weiler et al. in 2013, the MIC90 values were slightly different: AmB – 0.5 µg/mL, clotrimazole – 0.5 µg/mL, FCZ – 0.5 µg/mL, KTZ – 0.5 µg/mL, ITZ – 0.25 µg/mL, miconazole – 0.5 µg/mL, nystatin – 1 µg/mL, and VCZ – 0.125 µg/mL. In the study by Cafarchia et al. in 2012, the MIC90 values were: FCZ – 16 µg/mL, KTZ – 0.032 µg/mL, ITZ – <0.008 µg/mL, PSZ – 0.016 µg/mL, and VCZ – 0.064 µg/mL. Analysis of these results indicates that, despite some differences, the overall antifungal susceptibility profile of *M. pachydermatis* is relatively consistent. The lowest MIC90 values are observed for azoles such as itraconazole and posaconazole, indicating high *in vitro* activity, whereas fluconazole in the Álvarez-Pérez et al. (2016) study exhibited relatively high MIC values, suggesting probable resistance to this drug (Cafarchia et al. 2012, Weiler et al. 2013, Álvarez-Pérez et al. 2016). In another study by Nojo et al. the MIC90 values for eight isolates were: FCZ: 32 µg/mL; ITZ: 4 µg/mL; MCZ: >32 µg/mL (Nojo et al. 2025). Significant discrepancies in MIC90 values between studies, particularly for fluconazole, highlight the impact of methodological differences (e.g., type of medium, incubation time) on test results. This complicates setting of universal breakpoints and underscores the urgent need for standardization. Antifungal susceptibility testing conditions and reported MIC values (µg/mL) of *M. pachydermatis* across four independent studies are presented in Table 1.

Notably, these four studies employed different test conditions, which likely explain much of the observed variability in MIC90 values. Álvarez-Pérez et al. (2016) used the CLSI reference broth microdilution method with Sabouraud dextrose broth supplemented with 1% Tween 80 instead of standard RPMI 1640, incubating plates at 32°C and reading MICs macroscopically at 72 h, with an endpoint of 100% growth inhibition for amphotericin B and ≤90% inhibition for the remaining agents. Weiler et al. (2013) followed the CLSI M27-A3 protocol but used lipid-free RPMI 1640 broth, an atypical medium choice for this lipid-dependent yeast. Cafarchia et al. (2012) applied CLSI reference broth microdilution in parallel with the E-test method. Nojo et al. (2025) used a modified broth microdilution method in Sabouraud dextrose broth with 1% Tween 80, defining the MIC endpoint as 80% growth inhibition, a criterion that differs from the conventions applied in the other studies. These differences in growth medium, incubation conditions, and MIC endpoint definition substantially limit the comparability of MIC90 values across studies, particularly for fluconazole, and reinforce the urgent need for a standardized antifungal susceptibility testing protocol for *M. pachydermatis*.

Antifungal resistance mechanisms

Acquired resistance to antifungal drugs is an increasing challenge in the treatment of fungal infections, including those caused by *Malassezia*. The main factors promoting the development of resistance include improper drug use, prolonged therapy, exposure to environmental fungicides, and spontaneous mutations (Hossain et al. 2022).

Jesus et al. (2011) demonstrated *in vitro* that *M. pachydermatis* can acquire resistance to fluconazole following prolonged exposure to the drug. Using 30 fluconazole-susceptible clinical isolates, the authors generated a matched set of 30 fluconazole-resistant derivatives through *in vitro* induction. While the susceptible isolates showed low MICs to ketoconazole (0.01-1.0 µg/mL), itraconazole (0.01-1.0 µg/mL), voriconazole (0.01-4.0 µg/mL), and fluconazole (0.01-4.0 µg/mL), the induced fluconazole-resistant isolates displayed elevated MICs not only to fluconazole (64.0-128.0 µg/mL) but also to itraconazole (0.06-64.0 µg/mL), ketoconazole (0.25-32.0 µg/mL), and voriconazole (2.0-128.0 µg/mL), demonstrating clear cross-resistance to these three azoles. These findings underscore the necessity of performing susceptibility testing, which is a key component in selecting appropriate antifungal therapy in both human and veterinary medicine (Jesus et al. 2011).

Resistance mechanisms include modifications of the drug target proteins, overexpression of target enzymes, changes in membrane sterol composition, increased activity of efflux pumps, and activation of cellular stress response pathways (Cannon et al. 2009, Singh et al. 2009). For azoles, resistance primarily involves lanosterol 14α-demethylase (ERG11/cyp51), with mutations and gene overexpression leading to reduced fungal susceptibility, which has been confirmed in *M. pachydermatis*, including clinical and environmental isolates resistant to itraconazole and fluconazole (Kim et al. 2018, Peano et al. 2020, Díaz et al. 2023).

Kim et al. (2018) demonstrated that the region of chromosome 4 underwent a fourfold tandem duplication in two *M. pachydermatis* isolates, namely an environmental isolate with high MIC to ketoconazole and an *in vitro* mutant. This change increased the expression of genes in this region, including ERG11 and ERG4, which are involved in the metabolic pathway targeted

Table 1. Antifungal susceptibility testing conditions and reported MIC values (µg/mL) of *Malassezia pachydermatis* across four independent studies.

Studies	Álvarez-Pérez et al. (2016)	Weiler et al. (2013)	Cafarchia et al. (2012)	Nojo et al. (2025)
Number of strains	216 isolates from 28 cases (24 canine otitis, 3 canine dermatitis, 1 feline otitis);	80 total: G1: 40 otitis, G2: 40 healthy ear canals	62 (30 without lesions + 32 with skin lesions)	8 pre-selected azole-resistant isolates (+ CBS1879T reference strain)
Reference method	CLSI reference broth microdilution	CLSI M27-A3 broth microdilution	Modified CLSI M27-A2 broth microdilution	Modified broth microdilution
Culture medium	Sabouraud dextrose broth + 1% Tween 80	RPMI 1640 broth	Sabouraud dextrose broth + 1% Tween 80	Sabouraud's dextrose broth (1% peptone, 2% glucose) + 1% Tween 80
Incubation conditions	32°C, 72 h, macroscopic reading	37°C, 48 h	32°C, 48 h, visual reading	not reported
MIC endpoint definition	100% inhibition (AmB); ≤90% inhibition (other agents)	CLSI M27-A3	90% reduction in turbidity vs. growth control	80% growth inhibition (lowest concentration)
Minimum and maximum MIC values obtained (µg/mL)				
Amphotericin B	0.063-0.5	G1: 0.01-1 G2: 0.01-0.5	-	-
Fluconazole	4->64	G1: 0.01-1.0 G2: 0.01-0.5	4->64	>32 (all isolates)
Ketoconazole	0.125-4	G1: 0.01-0.5 G2: 0.01-0.5	0<0.008-0.060	-
Itraconazole	0.031-1	G1: 0.01-0.5 G2: 0.01-0.25	<0.008-0.016	0.125-16
Luliconazole	-	-	-	0.25-32
Posaconazole	0.125-4	-	<0.008-0.060	-
Voriconazole	0.063-8	G1: 0.01-0.25 G2: 0.01-0.125	0.030-0.500	-
Terbinafine	0.063-2	-	0.030-0.250	-
Clotrimazole	-	G1: 0.01-1 G2: 0.01-0.5	-	-
Miconazole	-	G1: 0.01-0.5 G2: 0.01-0.5	0.125-2	16->32
Nystatin	-	G1: 0.01-2 G2: 0.01-1	-	-

by ketoconazole (Kim et al. 2018, Domán et al. 2025). Peano et al. (2020) found that a clinically resistant miconazole isolate carried missense mutations in ERG11. Similar mutations were described in two environmental isolates showing multi-azole resistance *in vitro* and in CBS 1879 clones resistant to miconazole (Peano et al. 2020). Reduced azole susceptibility associated with these mechanisms is not confined to a single geographic region. Similar patterns of decreased fluconazole and itraconazole susceptibility have recently been reported among clinical *M. pachydermatis* isolates from Japan (Murayama and Kano 2023) and among *Malassezia* spp. isolates from Colombia (Galvis-Marín et al. 2025), confirming that reduced azole susceptibility in *M. pachydermatis* is a globally distributed phenomenon rather than an isolated regional finding.

In addition to target modification or overexpression, the dominant resistance mechanism involves enhanced drug efflux (Domán et al. 2025). In azole therapy, resistance often arises from increased activity of membrane pumps, which reduce intracellular drug concentration and limit efficacy (Cannon et al. 2009). In *Candida*

albicans, azole resistance is associated with overexpression of ABC transporters such as Cdr1 and Cdr2, and the MFS transporter Mdr1. Activating mutations in TAC1 cause constitutive overexpression of CDR1 and CDR2, while changes in MRR1 increase MDR1 expression (Lee et al. 2023). A similar defense mechanism has been observed in *M. pachydermatis*, including cross-resistance to fluconazole and other azoles. Cafarchia et al. (2015) suggested that fluconazole resistance may result from induction of efflux pumps encoded by multidrug resistance genes (e.g., MDR1), whereas cross-resistance to other azoles may be mediated by other genes such as CDR, similar to *Candida* species (Cafarchia et al. 2015). This was confirmed experimentally by combining fluconazole with haloperidol (HAL), an efflux pump inhibitor, which enhanced drug efficacy *in vitro* (MIC for 14 isolates: without HAL 8-512 µg/mL; with HAL 2-64 µg/mL) (Iatta et al. 2017).

Additionally, the similarity of fluconazole MIC values between *Malassezia* species and *Pichia kudriavzevii* (formerly *Candida krusei*) – a species with well-documented intrinsic resistance specifically to fluconazole while remaining susceptible to other azoles – suggests a probable intrinsic resistance mechanism to fluconazole, rather than to azoles as a class, in *Malassezia* (Cafarchia et al. 2015). This is consistent with the generally low MIC90 values observed for itraconazole, ketoconazole, posaconazole, and voriconazole across the studies discussed above.

Biofilm formation by *M. pachydermatis* has also been shown to significantly increase antifungal resistance, with 90% of biofilm-producing strains exhibiting resistance to fluconazole, miconazole, ketoconazole, itraconazole, posaconazole, terbinafine, and voriconazole (Figueredo et al. 2013). More recent data confirm and extend these findings: mature *M. pachydermatis* biofilms show near-complete resistance to itraconazole (100%), posaconazole (95.3%) and voriconazole (83.7%), whereas planktonic cells of the same isolates remain considerably more susceptible, underscoring biofilm formation as a major and persistent obstacle to successful azole therapy (Čonková et al. 2022).

Efficacy of essential oils

In the context of increasing fungal resistance to conventional antifungal drugs and the limitations of standard therapies, there is growing interest in alternative and innovative treatment strategies. Current research focuses on the use of plant-derived compounds, such as essential oils. These are complex mixtures of plant-derived compounds, mainly terpenoids and phenylpropanoids, exhibiting diverse biological properties, including antibacterial, antifungal, anti-inflammatory, and antioxidant effects (Dhifi et al. 2016, de Sousa et al. 2023). Their composition and activity depend on the plant species, chemotype, developmental stage, and environmental conditions. The antifungal activity of essential oils mainly results from their ability to disrupt cell membrane integrity, inhibit ergosterol and cell wall biosynthesis, and interfere with cellular metabolism, including mitochondrial function and transport pumps (Hammer et al. 2004, Nazzaro et al. 2017). Many essential oils also demonstrate synergistic effects with antifungal drugs, enhancing their efficacy against pathogens resistant to conventional therapies (Ahmad et al. 2013).

Some essential oils, including thyme (*Thymus vulgaris*), lemon (*Citrus limon*), roman chamomile (*Anthemis nobilis*), and basil (*Ocimum basilicum*), exhibit promising antimicrobial properties, making them potentially effective components in veterinary therapies aimed at treating skin and ear infections in animals. Additionally, the use of essential oil blends with different antifungal, anti-inflammatory, and skin-regenerative properties allows for a synergistic effect, enabling dose reduction while maintaining therapeutic efficacy. In *in vivo* studies conducted by Nardoni et al. (2017), good tolerance of certain essential oil blends and significant improvement in the clinical condition of animals was observed. The best results were achieved with a blend containing low concentrations of lemon and Roman chamomile oils. This blend showed high efficacy both *in vitro* and *in vivo*, inhibiting yeast growth at minimal concentrations while being well tolerated by all treated dogs. However, a blend containing citrus oils at a total concentration of 2% caused adverse reactions in two out of four dogs. These observations highlight the importance of appropriate selection and dosing of essential oils in veterinary practice. Although no significant reduction in blastospore counts was recorded after treatment, the improvement in skin and ear condition may reduce the need for conventional antifungal drugs, which is particularly relevant in the context of increasing drug resistance (Nardoni et al. 2017).

Numerous *in vitro* studies have shown that certain essential oils exhibit significant antifungal activity against *M. pachydermatis*. The most effective include oils from *Origanum vulgare* (oregano), *Syzygium aromaticum* (cloves), *Cinnamomum zeylanicum* (cinnamon), and *Satureja montana* (winter savory). In one study, the antifungal activity of 15 essential oils was evaluated against a reference strain of *M. pachydermatis* and several clinically isolated strains. Three concentrations were tested: 0.5%, 5%, and 30%. The lowest

concentration (0.5%) showed no inhibitory effect on yeast growth, indicating no activity at this low level. At 5%, only cinnamon and clove oils exhibited inhibitory effects, but only against 33-38% of the strains. Only at the highest concentration of oils from oregano, cloves, and cinnamon demonstrated strong and consistent antifungal activity against all tested isolates, suggesting their potential therapeutic application. Essential oils derived from lavender, bergamot, sage, tea tree, juniper, pine, grapefruit, cedar, or chamomile showed no significant activity against *M. pachydermatis* at any tested concentration. These results confirm that the efficacy of essential oils strongly depends on their chemical composition, particularly the presence of phenolic monoterpenes such as thymol, carvacrol, and eugenol (Váczi et al. 2018). Studies conducted by Bismarck et al. (2020) demonstrated antifungal activity of selected essential oils against *M. pachydermatis*. Oils from *Satureja montana* (winter savory), *Cymbopogon citratus* (lemongrass), *Origanum vulgare* (oregano), *Cymbopogon martinii* (palmarosa), and cinnamon leaves were particularly effective (Bismarck et al. 2020). One study reported promising *in vitro* activity of lavender, lemon, and oregano essential oils against *M. pachydermatis*. The study included five clinical isolates from cases of canine otitis externa and one reference strain. Oregano oil showed the highest activity, while lemon and lavender oils, although demonstrating antimicrobial activity in microdilution assays, did not inhibit growth in agar-based tests at diluted concentrations (Pistelli el al. 2012). Cinnamon essential oil exhibited strong activity against 20 *M. pachydermatis* isolates obtained from dogs with otitis externa, with low MIC values (0.008%), indicating high efficacy (Sim et al. 2019).

In recent years, an increasing number of researchers have focused on the use of essential oils in veterinary therapies and in cosmetic products for animals, recognizing their natural anti-inflammatory, antibacterial, and care-promoting properties. Essential oils show promising potential in veterinary therapy, particularly in the treatment of fungal infections of the skin and ear canal in dogs caused by *Malassezia* yeasts. *In vivo* studies evaluating the efficacy and safety of the product Malacalm, containing a mixture of six essential oils: 1% bitter orange (*Citrus aurantium*), 1% lavender (*Lavandula officinalis*), 0.5% oregano (*Origanum vulgare*), 0.5% marjoram (*Origanum majorana*), 0.5% peppermint (*Mentha piperita*), and 0.5% immortelle (*Helichrysum italicum*), dissolved in sweet almond and coconut oils, demonstrated that the Malacalm essential oil mixture can represent an effective and safe alternative to conventional dermatological therapies in dogs with skin infections caused by *Malassezia* yeasts. In 90% of dogs treated with Malacalm, complete resolution of clinical signs was observed, while the remaining animals showed moderate improvement. Importantly, during long-term follow-up (180 days), no recurrence of symptoms was recorded in the group treated with the essential oil mixture, in contrast to the control group receiving conventional treatment, in which all patients experienced relapse. Additionally, the efficacy was not limited to reducing yeast numbers but also included improvement in the overall condition of the skin and ear canal (Nardoni et al. 2014).

In a study conducted by Schlemmer et al. (2019), the *in vitro* activity of selected essential oil components (carvacrol, cinnamaldehyde, and thymol) was evaluated both as monotherapy and in combination with conventional antifungal drugs (such as fluconazole, itraconazole, ketoconazole, clotrimazole, miconazole, terbinafine, and nystatin) against *M. pachydermatis*. Based on the mean fractional inhibitory concentration index (FICI), synergistic effects were demonstrated for the combinations: nystatin with carvacrol, nystatin with thymol, and miconazole with carvacrol (Schlemmer et al. 2019). Additionally, Bohmova et al. (2019) reported synergistic interactions between clotrimazole and tea tree, peppermint, and oregano oils (Bohmova et al. 2019). Another study showed that essential oils from oregano, thyme, and rosemary exhibit high efficacy against *M. pachydermatis*. The strongest activity was observed for oregano oil, attributed to the presence of carvacrol. The addition of chlorhexidine enhanced the activity of all tested oils, indicating a synergistic effect. Cinnamon and clove oils also displayed antifungal activity but required higher concentrations. Bergamot oil showed the weakest activity, although additive or synergistic effects were observed when combined with chlorhexidine (Váczi et al. 2024).

Recent studies of Čonková from 2025, conducted on 15 clinical isolates of *M. pachydermatis*, demonstrated high antifungal efficacy of coriander essential oil, as well as oils derived from nutmeg, bergamot, Spanish sage, hyssop, and rosemary. In contrast, essential oils from grapefruit, lavender, tea tree, and oregano exhibited lower antifungal activity. The observed discrepancies compared to other literature reports may result not only from differences in the chemical composition of essential oils but also from the lack of standardized protocols for determining MIC values of essential oils and from the limited comparability of methodologies used in studies on *M. pachydermatis*. (Čonková et al. 2025)

The content of active compounds in natural essential oils can vary depending on the region and season of harvest. In the study by Nojo et al. (2025), the anti-Malassezia activity of three essential oil components carvacrol, citral, and thymol was evaluated. Ana-

lysis of eight azole resistant strains showed an activity range of 0.03-0.125% for all three compounds. Given the variability of natural essential oils, the use of industrially synthesized phytochemicals offers greater stability and lower cost. (Nojo et al. 2025) These results suggest that combining selected essential oils or their components with conventional antifungal drugs may enhance their efficacy against *M. pachydermatis*, providing potential for the development of new therapeutic strategies.

Essential oils are not the only alternative approach being explored for the management of *M. pachydermatis* infections. Other non-conventional strategies, including drug repositioning, novel ear-cleaning formulations, therapeutic diets, and physical modalities such as cold atmospheric plasma, have also shown promise. In particular, cold atmospheric microwave plasma demonstrated synergistic antifungal activity against *M. pachydermatis* when combined with chlorhexidine gluconate (Lee et al. 2022), while a recent comprehensive review summarized the full spectrum of alternative treatment options for canine *Malassezia otitis* externa beyond conventional antifungal drugs (Gomes et al. 2024). Together, these findings indicate that the search for adjunctive or alternative antifungal strategies extends well beyond essential oils, reflecting the broader urgency of addressing antifungal resistance in *M. pachydermatis*.

Summary and perspectives

M. pachydermatis maintains its position as a significant opportunistic cutaneous pathogen in companion animals. As demonstrated in this review, although this species is a natural commensal, under favorable conditions it activates complex pathogenic mechanisms, leading to clinically burdensome dermatoses and otitis externa. Despite well-established knowledge of its taxonomy and biology, substantial challenges persist in clinical practice. A key limitation remains the lack of standardization in antifungal susceptibility testing. Discrepancies in MIC90 values for the same drugs reported in different studies clearly indicate that result comparison is unreliable without harmonization of culture protocols, media, and interpretative criteria. The development of clinical breakpoints for *M. pachydermatis* by organizations such as EUCAST or CLSI is essential for rational therapy and resistance trend monitoring.

The growing problem of multidrug resistance, particularly to azoles, is driven by mechanisms such as overexpression and mutations in the *ERG11* gene, increased efflux pump activity, and the ability to form biofilms. In the context of these phenomena, conventional pharmacotherapy may be insufficient.

The solution to this challenge seems the application of essential oils. *In vitro* studies have confirmed the high efficacy of oregano and clove oils – preliminary *in vivo* reports indicate their potential as standalone agents or synergists enhancing the activity of conventional drugs. Despite these encouraging results, further rigorous clinical studies are required before their implementation in practice, addressing not only efficacy but also dose standardization, long-term safety, and adverse effect profiles across different animal species and breeds. This review provides a novel integrative perspective by linking molecular resistance mechanisms with diagnostic limitations and emerging alternative therapies, thereby contributing to a more comprehensive understanding of therapeutic challenges and supporting the development of more effective and targeted treatment strategies.

Author Declarations

Ethics approval

This article is a review paper and does not involve studies with human participants or animals. Therefore, ethical approval from a bioethics committee was not required.

Use of generative artificial intelligence

The authors used generative artificial intelligence tools solely to assist with the linguistic and stylistic editing of the text. These tools were not used to generate scientific content, analyze data, or interpret results. The final content of the manuscript was prepared and verified by the authors.

Conflict of interest

The authors declare no conflict of interest.

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