In Vitro Reduction of Antifungal Azole Sensitivity in *Malassezia spp.* and Resistance Prevention Strategies

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Abstract

Malassezia spp. are lipophilic yeasts that constitute part of the normal skin flora but are also implicated in various dermatological conditions, including pityriasis versicolor, seborrheic dermatitis, and folliculitis. In recent years, decreased in vitro antifungal susceptibility has been increasingly reported, potentially contributing to therapeutic failure and disease recurrence. Literature evaluating the minimum inhibitory concentration (MIC) patterns of azole agents and the molecular mechanisms of the decreased susceptibility in Malassezia spp. remains limited. Therefore, this literature review examines scientific articles related to the antifungal susceptibility of commonly used azoles against skin infections caused by Malassezia spp. Relevant publications were analyzed, focusing on in vitro studies, minimum inhibitory concentration (MIC) values, resistance mechanisms, and strategies for preventing antifungal resistance. Several studies demonstrate increased MIC values, particularly for azole antifungals such as fluconazole and ketoconazole. Fluconazole exhibited the highest MIC values (4 to >64 mg/L; MIC50 32 mg/L; MIC90 64 mg/L), whereas posaconazole showed the lowest (0.06–2 mg/L; MIC50 0.125 mg/L; MIC90 0.5 mg/L). Ketoconazole and itraconazole demonstrated relatively good antifungal activity, while amphotericin B showed moderate efficacy. Compared to Candida spp., Malassezia spp. generally exhibited higher MIC values, suggesting reduced susceptibility. Resistance mechanisms include ERG11 gene mutations, increased efflux pump activity, and biofilm formation. Decreased antifungal susceptibility in Malassezia spp. represents an emerging clinical concern with significant implications for treatment outcomes. Rational therapeutic approaches, including antifungal stewardship, susceptibility-guided therapy, and combination strategies, are essential to prevent resistance and improve clinical effectiveness.

Keywords: *Azoles, Drug Susceptibility, Malassezia, Minimum Inhibitory Concentration.*

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A. INTRODUCTION

Malassezia is a genus of lipophilic fungi that is physiologically part of the normal flora of human skin, but plays a crucial role in the pathogenesis of various common skin diseases. Diseases associated with Malassezia include seborrheic dermatitis, pityriasis versicolor, and Malassezia-associated folliculitis. To date, there are no cumulative estimates representing the total global prevalence of Malassezia infections. However, epidemiological evidence suggests that pityriasis versicolor may affect up to 20–25% of the world's population, particularly in tropical regions, while seborrheic dermatitis has a reported global prevalence of approximately 4.38% (Hamdino et al., 2022). These findings confirm that Malassezia-associated skin diseases are among the most common dermatological conditions globally. Antifungal

therapy against Malassezia is one of the most frequently used interventions in daily clinical practice (Rhimi et al., 2021).

For decades, Malassezia infections and related conditions were considered relatively easy to treat. Azole antifungals, both systemic and topical, such as ketoconazole and miconazole, have demonstrated good clinical efficacy (Wang et al., 2020). However, in recent years, reports have emerged of decreased therapeutic response, frequent relapses, and the need for repeated treatments, particularly in seborrheic dermatitis and pityriasis versicolor. This phenomenon raises questions about the possibility of decreased antifungal sensitivity in Malassezia. Unlike dermatophytes and Candida, antifungal drug resistance in Malassezia has not been clearly described in clinical guidelines. This is due to the lack of standard methods for culturing Malassezia, which is lipophilic and requires special nutrients, and the lack of standard MIC values for determining Malassezia sensitivity to azole antifungals. Nevertheless, various in vitro studies of clinical isolates of Malassezia obtained from patients with skin diseases and healthy skin have shown increased minimum inhibitory concentration (MIC) values to several azole drugs, particularly ketoconazole and fluconazole (Chebil et al., 2022).

Decreased antifungal sensitivity to Malassezia can exacerbate disease severity, leading to chronicity and higher relapse rates. Seborrheic dermatitis, for example, is a highly prevalent, chronic-recurrent disease that requires long-term therapy. Failure or decreased antifungal efficacy not only leads to prolonged fungal infections but also prolongs the inflammatory skin process, worsening symptoms and reducing the patient's quality of life (Walyatalattof et al., 2025). Furthermore, Malassezia has unique biological characteristics compared to other pathogenic fungi. This fungus relies on exogenous lipids for growth and exhibits a wide diversity of species, including M. globosa, M. restricta, and M. furfur, each of which has a distinct antifungal sensitivity profile (Saunte et al., 2020). This variation has the potential to influence therapeutic response and contribute to the phenomenon of tolerance or decreased drug sensitivity.

The widespread, repeated, and often unconfirmed use of systemic and topical azoles can create an environment that favors the selection of strains with lower antifungal sensitivities. Although clinical evidence of resistance is still limited, this pattern is similar to the initial mechanism of resistance emergence in other fungi, which begins with decreased sensitivity (Galvis-Marrin et al., 2025). To date, azole drugs remain the mainstay of therapy, while other antifungal classes such as allylamines or echinocandins have a limited role, narrow spectrum, and are not cost-effective in the treatment of dermatological diseases. This situation has prompted the need for new resistance prevention and therapeutic strategies, including optimizing therapy regimens, drug combination approaches, and adding adjuvants with antifungal and anti-inflammatory activity (Pedrosa et al., 2019). Therefore, the recent development of decreased antifungal sensitivity to Malassezia is a crucial issue in dermatology. Discussion of this topic is not only relevant for understanding the causes of therapy failure and disease recurrence, but also provides a basis for developing more rational, sustainable, and evidence-based therapeutic approaches in the future.

The purpose of writing this paper is to increase the reader's insight into the latest developments regarding the reduction of azole antifungal sensitivity to Malassezia and resistance prevention strategies.

B. LITERATURE REVIEW

1. Immunopathogenesis of Malassezia Infection

Malassezia spp. physiologically colonizes human skin, particularly in areas rich in sebum. Under normal conditions, Malassezia spp. are commensal and tolerated by the skin's immune system. However, in certain individuals, a shift in the host-microorganism interaction occurs, causing Malassezia spp. to trigger a pathological immune response. This transition is not solely determined by the number of fungi, but by a complex interaction between Malassezia spp. virulence factors, skin barrier integrity, and the host's innate and adaptive immune responses. Malassezia spp. metabolic products derived from lipid degradation can irritate the skin and trigger activation of the innate immune system (Sparber & LeibundGut-Landmann, 2017).

In the innate immune response, Malassezia spp. are recognized by keratinocytes and skin immune cells through pattern recognition receptors (PRRs), including Toll-like receptors (TLR2, TLR4) and C-type lectin receptors. Activation of this pathway triggers the production of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α, as well as inflammasome activation. Inflammasome activation plays a key role in the pathogenesis of seborrheic dermatitis and other inflammatory conditions associated with Malassezia. Furthermore, activated dendritic cells facilitate the differentiation of adaptive immune responses, including the activation of helper T cells (Th1, Th17, and Th2), which contribute to chronic and recurrent skin inflammation.

In the adaptive immune response, Malassezia is immunogenic and can maintain skin inflammation through the activation of memory T cells and the long-term release of inflammatory cytokines. Thus, the immunopathogenesis of Malassezia infection is not solely due to fungal colonization but rather the result of an uncontrolled host immune response. This explains the frequent chronic, recurrent, and inflammatory nature of Malassezia-associated diseases, even when fungal populations are relatively low (Piacentini et al., 2025).

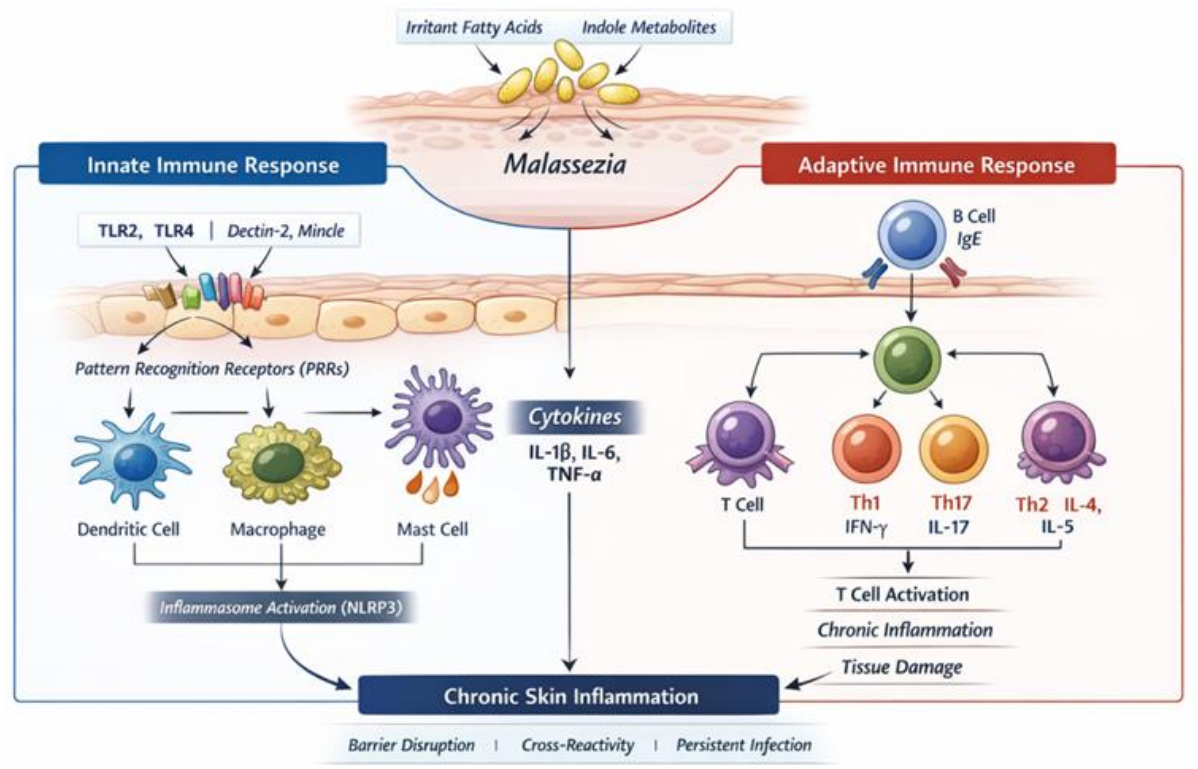

Figure 1. Immunopathogenesis of Malassezia spp. infection.

2. Pityriasis Versicolor

This infection is globally recognized as one of the most common dermatomycoses, particularly in tropical and subtropical countries, although epidemiological data are limited because it is often considered mild and underreported. Large population studies indicate that this disease is a common clinical problem that requires prompt diagnosis and a consistent therapeutic approach, particularly in endemic areas with hot and humid climates (Sparber et al., 2020).

Clinically, pityriasis versicolor (PV) is characterized by fine-scale macules or plaques with varying skin discoloration, including hypopigmentation, hyperpigmentation, or erythema. The characteristic lesions are usually found on the trunk, neck, and upper arms and are often not accompanied by intense itching, although some patients report mild pruritus. Diagnosis is generally made clinically with the aid of microscopic examination of KOH preparations demonstrating short hyphae and clustered spores ("spaghetti and meatballs"), as well as dermoscopic or Wood's lamp examination.

The management of pityriasis versicolor emphasizes the use of topical antifungals as first-line therapy, including imidazoles (ketoconazole, clotrimazole), allylamines, or ciclopirox olamine, applied for 1–4 weeks to eliminate Malassezia colonies. Recent evidence suggests that topical therapy is effective with a good safety profile, while systemic antifungals (itraconazole or fluconazole) are considered in cases that are extensive, severe, recurrent, or unresponsive to topical therapy. Relapse

prevention through patient education about trigger factors and hygiene habits is also important due to high relapse rates, reported as high as 80% in some cohorts.

3. Malassezia folliculitis

Chronic inflammation of Malassezia folliculitis occurs in the pilosebaceous unit. Clinical manifestations include monomorphic erythematous papules or perifollicular pustules approximately 2–3 mm in diameter. The distribution of lesions differs by gender. In men, lesions are commonly found on the back, shoulders, upper arms, chest, and neck, while in women, the most common sites of involvement include the face, shoulders, chest, and back (Chalupczak & Lipner, 2025). Pruritus is more common than in pityriasis versicolor. This condition can coexist with other skin conditions, such as recalcitrant acne vulgaris, seborrheic dermatitis, and pityriasis versicolor. Several predisposing factors include immune disorders such as diabetes mellitus, increased sebaceous gland activity during pregnancy, changes in skin flora due to long-term antibiotic use, and immunocompromised conditions such as hematologic malignancies (leukemia, Hodgkin's disease), post-organ transplantation (kidney, heart, or bone marrow), AIDS, and Down syndrome.

Diagnosis can be confirmed through microscopic examination using 20% KOH solution, with specimens obtained from the contents of pustules, papules, or comedonal papules, obtained through a comedone extraction procedure. A positive result is obtained if the result is +3 or +4 based on the grading of the number of spores per large field of view of the microscope. Jacinto-Jamora spore grading:

a. +1: 1-2 spores scattered, not in clusters
b. +2: 2-6 spores in clusters or 3-12 spores scattered
c. +3: 7-12 spores in clusters or 13-20 spores scattered
d. +4: >12 spores in clusters or >20 spores scattered

Several medications can be selected according to the following indications:

a. Topical therapy: Ketoconazole 2% shampoo twice a week for 2–4 weeks (1C), or Selenium sulfide 2.5% shampoo once a day for 3 days. Maintenance dose once a week. (1A)
b. Systemic therapy: Itraconazole 200 mg/day for 2–3 weeks (1A), Fluconazole 150 mg/week for 2–4 weeks (1C), or Ketoconazole 200 mg/day for 2–4 weeks (1A).

4. Seborrheic Dermatitis

Seborrheic dermatitis (SD) is a common chronic inflammatory skin condition with a papulosquamous morphology. It occurs in areas of the skin with numerous sebaceous glands, such as the face, scalp, ears, upper trunk, and flexures (inguinal, inframammary, and axillary). Seborrheic dermatitis exhibits a bimodal pattern of occurrence, divided into infantile seborrheic dermatitis (ISD) and adult seborrheic dermatitis (AD) (Galizia et al., 2024).

In the infantile variant, the condition usually appears within the first two weeks of life and generally resolves within 4–6 months. This condition, known as cradle cap, is characterized by oily, yellowish scales and rarely causes pruritus. In infants, the

lesions are non-itchy, oily, white to yellowish scales, primarily confined to the scalp but can also involve the retroauricular area and eyebrows. Involvement of the flexure folds, diaper area, and face is relatively rare. In contrast, in adolescents and adults, common complaints include erythema and scaling of the scalp, nasolabial folds, eyebrows, retroauricular area, forehead, and chest. Lesions less frequently affect the umbilicus, interscapularis, perineum, and anogenital area. Reddened skin areas are generally itchy, and patients often complain of dandruff (pityriasis sicca). Clinical manifestations can worsen with psychological stress or exposure to cold weather and tend to be chronic or recurrent.

Treatment for non-scalp seborrheic dermatitis in adults can be administered singly or in combination, depending on the severity. In mild cases, primary options include topical antifungals such as ketoconazole 2% or ciclopirox 1%, non-steroidal anti-inflammatory agents with antifungal properties (AIAFp), low-potency topical corticosteroids (1% hydrocortisone), and topical calcineurin inhibitors (pimecrolimus or tacrolimus), which are generally applied twice daily for four weeks. In moderate to severe cases, therapy can be enhanced with the use of medium-potency topical corticosteroids, such as desonide 0.05% cream or aclomethasone 0.05% ointment, applied twice daily for four weeks. In certain circumstances, especially when the response to topical therapy is inadequate, systemic antifungals may be considered, such as itraconazole at a dose of 200 mg per day for one week, followed by 200 mg per day for two days every month for up to eleven months, or terbinafine 250 mg per day given continuously for four to six weeks or intermittently for twelve days per month for three months.

Treatment for scalp seborrheic dermatitis in adults is based on severity and can be either monotherapy or combination therapy. Mild cases are generally treated with topical antifungals such as ketoconazole 1-2% shampoo or ciclopirox 1-5% applied two to three times per week, as well as alternatives such as AIAFp (piroctone olamine/bisabolol/glycyrrhetic acid/lactoferrin shampoo), keratolytic agents or tar, selenium sulfide, and zinc pyrithione. In certain circumstances, a mild to moderate potency topical corticosteroid in the form of a lotion or ointment may be added for a limited period. Moderate to severe cases require a high-potency topical corticosteroid (fluocinolone acetonide 0.01%) in shampoo form for 2 weeks, and if the response is inadequate, systemic antifungals such as itraconazole 200 mg/day for 1 week, then 200 mg/day for 2 days/month for 1-1 months, or fluconazole 50 mg/day for 2 weeks or 200–300 mg/week for 2–4 weeks, may be considered. To prevent relapse, maintenance therapy using ketoconazole shampoo or ciclopirox once per week for several months is recommended.

Figure 2. Management Flowchart for Seborrheic Dermatitis in Adults

In infants, seborrheic dermatitis treatment depends on the location of the lesions. On the scalp, therapy can include topical antifungals such as 2% ketoconazole shampoo applied twice weekly for four weeks, along with an emollient such as white petrolatum ointment for daily use to soften the scales. Another alternative is AIAFp in the form of a cream containing piroctone olamine, alglycerol, and bisabolol applied every twelve hours. Meanwhile, on non-scalp areas, therapy generally includes 2% ketoconazole cream applied once daily for seven days, and if necessary, a mild topical corticosteroid, such as 1% hydrocortisone cream, can be added for a short duration.

C. METHOD

This study employed a qualitative approach using a literature review design to synthesize and critically analyze the current evidence regarding reduced azole antifungal susceptibility in Malassezia species and its implications for resistance prevention strategies. This approach was chosen to provide a comprehensive understanding of recent developments by integrating findings from published scientific literature. Relevant data were obtained from peer-reviewed journal articles, review papers, clinical studies, and other scholarly publications related to Malassezia, azole antifungal susceptibility, mechanisms of resistance, and therapeutic strategies. Priority was given to recent publications while including seminal studies that remain highly relevant to the topic. After the relevant literature had been collected, the selected sources were systematically reviewed, categorized, and analyzed to identify consistent findings, emerging trends, and existing knowledge gaps. The collected data were then synthesized through descriptive qualitative analysis to explore the current evidence regarding decreased antifungal susceptibility, the potential mechanisms underlying this phenomenon, and strategies to prevent the development of antifungal

resistance. The findings of this review are expected to provide an evidence-based overview that may support clinicians and researchers in developing more rational and sustainable therapeutic approaches for Malassezia-associated skin diseases.

D. RESULT AND DISCUSSION

1. Mechanism of Action of Antifungals Against Malassezia Infections

Treatment of Malassezia-associated skin infections and conditions currently relies on antifungal agents that target the structure and function of fungal cells. Unlike other pathogenic fungi, Malassezia has unique biological characteristics, including a dependence on exogenous lipids and a unique cell membrane composition, which can lead to variations in response to antifungals across species. Understanding the mechanisms of action of each antifungal class is crucial to explaining therapeutic efficacy, limited effectiveness, and the phenomenon of decreased sensitivity reported in in vitro studies.

Azoles are the first-line and most widely used therapy for the treatment of Malassezia-associated skin conditions, both topically and systemically. Azoles work by inhibiting the enzyme lanosterol 14-α-demethylase (CYP51), a key enzyme in the biosynthesis of ergosterol, a major component of fungal cell membranes. Inhibition of this pathway leads to decreased ergosterol levels and the accumulation of toxic sterols, which disrupt the integrity and permeability of fungal cell membranes. In Malassezia, this mechanism has proven effective, but several in vitro studies have shown an increase in the minimum inhibitory concentration (MIC) of certain azoles, particularly ketoconazole and fluconazole, which is thought to be related to cell membrane adaptation and drug tolerance mechanisms (Leong et al., 2021).

Polyenes, such as amphotericin B, work by a different mechanism: they bind directly to ergosterol in the fungal cell membrane and form transmembrane pores. This pore formation causes leakage of electrolytes and intracellular molecules, leading to fungal cell death. In vitro, Malassezia spp. generally exhibit good sensitivity to amphotericin B. However, in dermatological practice, the use of polyenes is limited due to potential systemic toxicity and the limitations of topical formulations, making their role in Malassezia infections relatively minor compared to azoles.

Allylamines, such as terbinafine, work by inhibiting squalene epoxidase, a key enzyme in the ergosterol synthesis pathway. This inhibition leads to the accumulation of squalene, which is toxic to fungal cells, and a decrease in membrane ergosterol. Although this mechanism is highly effective against dermatophytes, the effectiveness of allylamines against Malassezia is more variable. Some studies indicate that Malassezia has a lower sensitivity to terbinafine than to azoles. This is likely related to differences in lipid metabolism and the sterol biosynthesis pathway in this genus. Therefore, allylamines are not the primary therapy for Malassezia-associated skin diseases (Billamboz & Jawhara, 2023).

Echinocandins work by inhibiting the synthesis of β-1,3-D-glucan, an essential component of fungal cell walls. However, Malassezia spp. have a relatively low β-1,3-D-glucan content in their cell walls, so echinocandins exhibit minimal or inconsistent

activity against this fungus. This is the reason why echinocandins are not recommended for the treatment of Malassezia infections, both in clinical practice and in dermatology guidelines. Understanding these mechanistic limitations reinforces the importance of selecting antifungals that are appropriate to the biological characteristics of Malassezia (Osset-Trénor et al., 2023).

Overall, differences in antifungal mechanisms of action and the biological characteristics of Malassezia contribute to the variation in therapeutic responses seen clinically. In vitro findings regarding increased MICs and heterogeneity in sensitivity between species emphasize that antifungal efficacy is determined not only by the drug class but also by the complex interactions between the fungus, the drug, and the skin environment. This provides a scientific basis for rationalizing antifungal use and developing more optimal therapeutic strategies in dermatology practice.

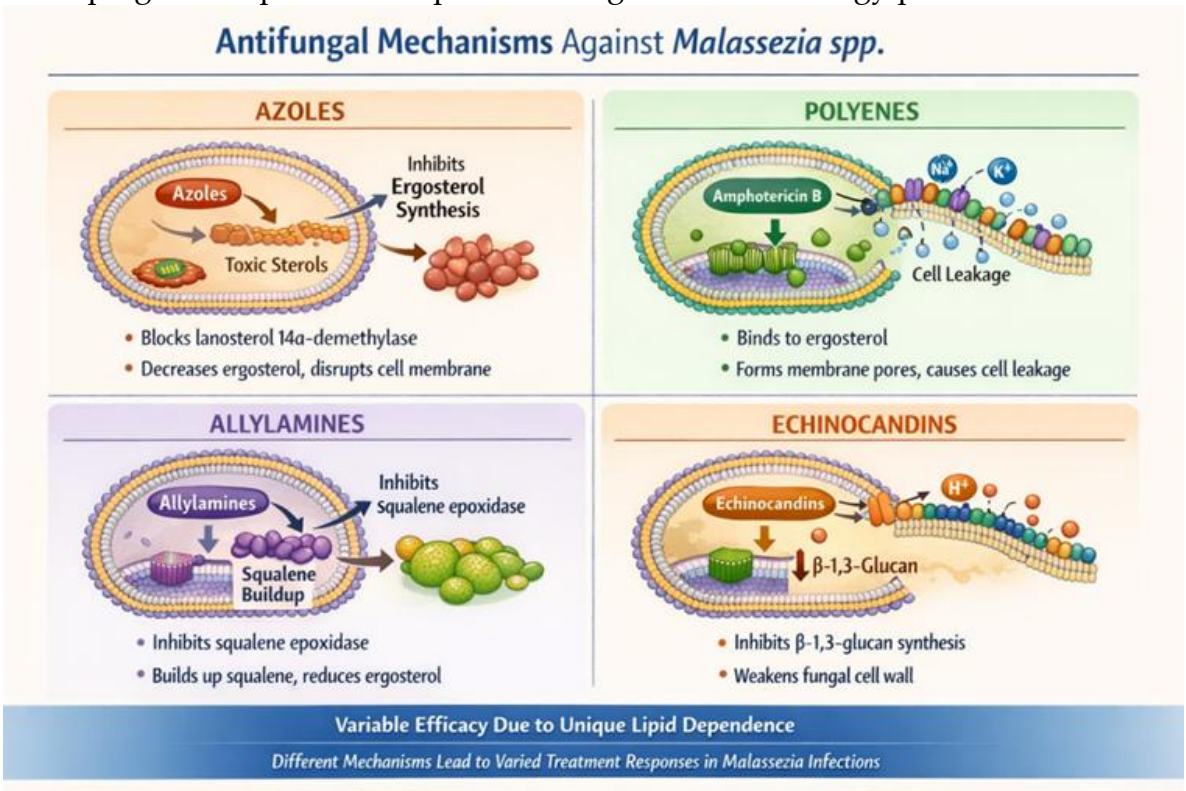

Figure 3. Antifungal mechanism against *Malassezia spp.*

2. In Vitro Antifungal Sensitivity Test Against Malassezia

In vitro antifungal susceptibility testing for Malassezia essentially follows the standard broth microdilution method recommended by the Clinical and Laboratory Standards Institute in document CLSI M27 for yeast. This method aims to determine the minimum inhibitory concentration (MIC), the lowest concentration of antifungal capable of inhibiting fungal growth. The basic principle of the test is to expose a fungal suspension to various concentrations of antifungal in a liquid medium, then assess growth after incubation. Standardization includes media composition, inoculum concentration, incubation temperature, and the method of reading results, so that test

results can be compared between laboratories and used as a basis for therapy selection (Alexander, 2017).

When applied to Malassezia, the CLSI method is modified due to the lipophilic nature of this organism. The procedure begins with the isolation of Malassezia colonies from the culture, then a suspension is prepared in sterile saline, and the turbidity is adjusted (0.5 McFarland equivalent) before being diluted to a final density of approximately 1–5 × 10^5 cells/mL. This suspension is then inoculated into a 96-well microtiter plate containing lipid-enriched liquid media and serial dilutions of antifungals (e.g., ketoconazole, itraconazole, fluconazole). It is then incubated at 32–35°C for approximately 48–72 hours. After incubation, fungal growth is observed visually or spectrophotometrically, and the MIC is determined as the lowest concentration that shows ≥50% growth inhibition compared to the control (Leong et al., 2017).

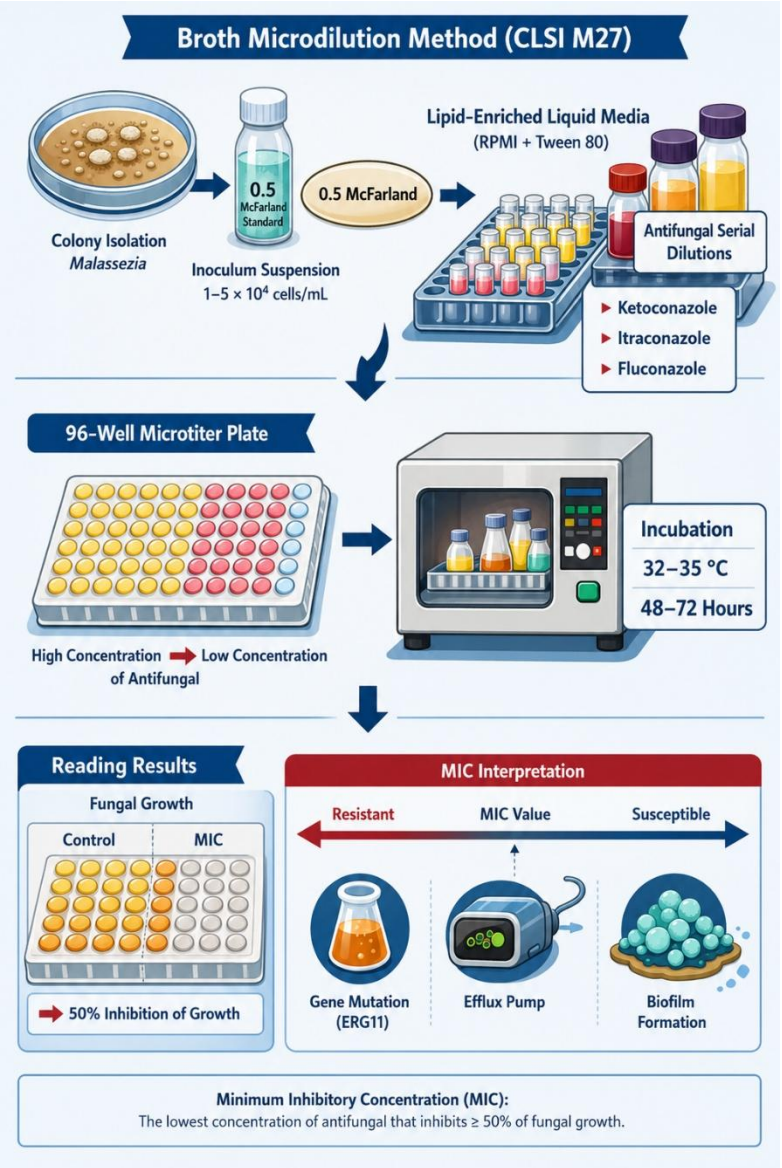

Figure 4. Broth Microdilution Method in Determining the MIC Value of Antifungal

Mechanistically, this test demonstrates the interaction between antifungals and their biological targets, such as inhibition of ergosterol synthesis by azoles or disruption of cell membrane integrity. High MIC values indicate decreased sensitivity and potential resistance, which can be caused by mutations in target genes (e.g., ERG11), increased efflux pump activity, or biofilm formation. In vitro susceptibility testing is particularly important in cases of chronic, recurrent, or recalcitrant Malassezia infections, as it helps clinicians choose more rational therapy while preventing antifungal resistance. However, there is currently no CLSI standard that is fully specific for Malassezia, so interpretation of results must still be related to the patient's clinical condition.

3. Mechanisms of Fungal Resistance to Antifungals

Malassezia virulence factors include lipase, phospholipase, and acid sphingomyelinase, which contribute to the degradation of sebum lipids into fatty acids that support Malassezia growth and cause skin disease. Cell wall resistance, melanin, reactive oxygen species, hyphal formation, and hydrophobicity are involved in modulating the immune response and contribute to Malassezia pathogenesis. The molecular mechanisms of antifungal resistance in Malassezia spp. show similarities to those in other pathogenic yeasts, such as Candida spp. One of the main mechanisms of azole resistance in Malassezia is changes in the target enzyme lanosterol 14α-demethylase encoded by the ERG11 gene. Missense mutations and increased copy number of the ERG11 gene have been reported to play a role in reducing the affinity of azoles for the target enzyme without disrupting the physiological function of ergosterol biosynthesis, allowing the fungus to survive under drug pressure (Park et al., 2020). In addition, the expression of efflux pumps is also an important mechanism contributing to the decrease in azole sensitivity in Malassezia. The activity of these efflux pumps leads to a decrease in intracellular antifungal concentrations and has been demonstrated through a decrease in MIC values after the use of efflux inhibitors (Deegan et al., 2019).

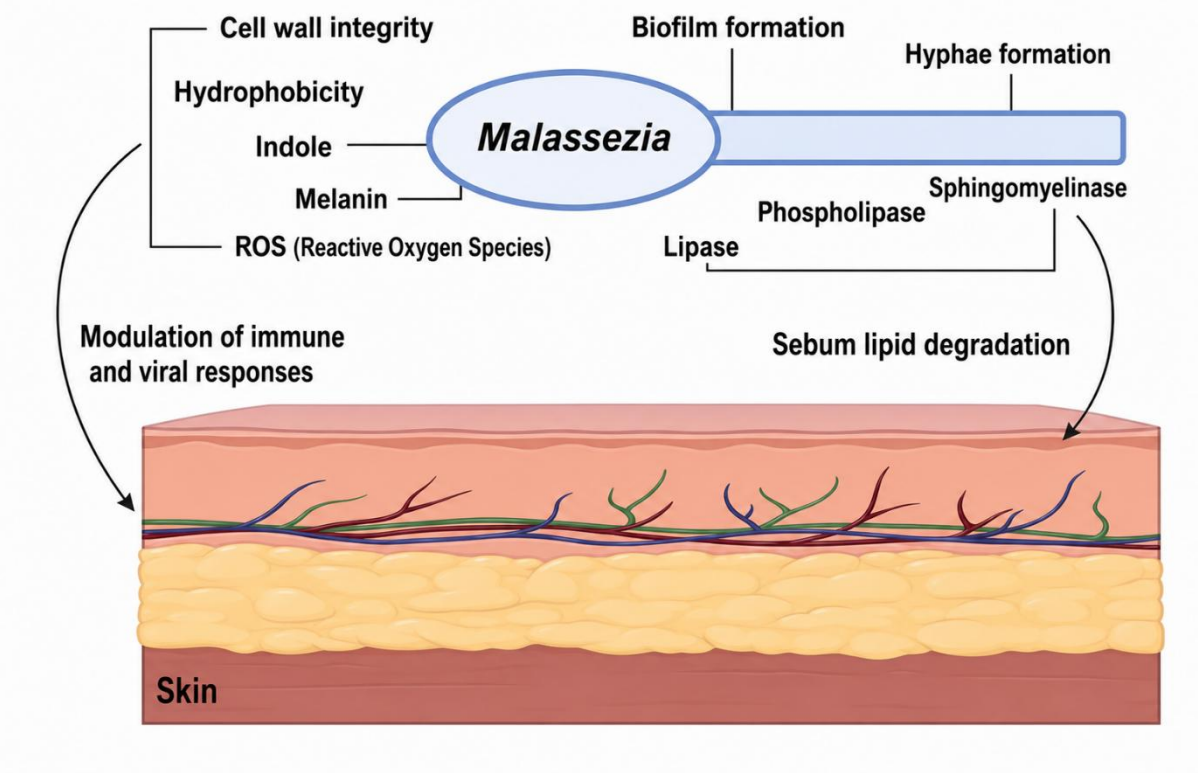

Figure 5. Malassezia Virulence Factors

In addition to genetic mechanisms and drug transport, biofilm formation also contributes significantly to antifungal tolerance in Malassezia. Biofilms create a microenvironment that protects fungal cells from antifungal penetration and enhances the expression of genes that promote decreased drug sensitivity. Therefore, Malassezia isolates that form biofilms tend to exhibit lower sensitivity to azoles. This combination of mechanisms suggests that decreased antifungal sensitivity in Malassezia is adaptive, posing a challenge in the management of skin diseases associated with this fungus (Leong et al., 2021).

Studies of Malassezia cells cultured in microplates and forming biofilms (as in pathological conditions) showed that MIC values were consistently higher than those of Malassezia cells as normal, non-colonizing (planktonic) flora. The most pronounced MIC increase was observed for fluconazole, indicating that biofilm formation plays a significant role in reducing the effectiveness of this drug. Interestingly, several isolates previously categorized as sensitive in the planktonic form changed to non-sensitive after forming biofilms. These findings suggest that decreased sensitivity of Malassezia spp. is significantly influenced by biofilms.

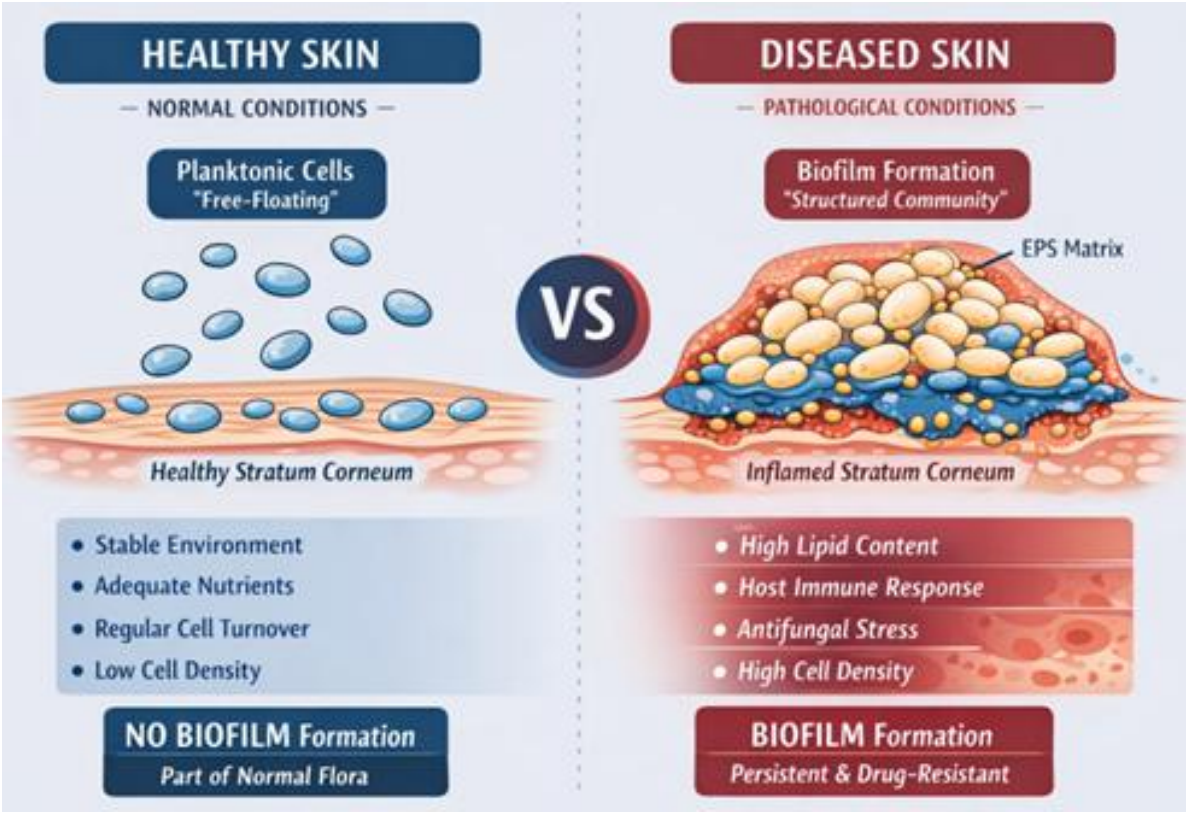

Figure 6. Comparison of Malassezia in healthy skin and pathological conditions

Fungi possess a defense mechanism in the form of efflux pumps, membrane proteins that function to expel antifungal drugs from the cell. This activity can reduce the intracellular concentration of the drug, making the exposure time insufficient to produce optimal effects, which phenotypically manifests as resistance. In one study, a combination test was conducted comparing exposure to a single azole and an azole combined with an efflux pump inhibitor (which inhibits efflux pump function, preventing the drug from entering the cell and being immediately excreted).

The decrease in MIC values after the addition of an efflux pump inhibitor indicates that the efflux mechanism plays a role in reducing the concentration of the azole drug, inhibiting the growth of Malassezia spp. This was evident for itraconazole, ketoconazole, and voriconazole. In contrast, for fluconazole, the addition of an inhibitor did not reduce the MIC and, in some isolates, even increased it. These findings indicate that efflux pumps are not the primary mechanism of decreased fluconazole sensitivity, and that other mechanisms may be involved, such as biofilm formation, changes in the drug's target of action, or other transport systems unaffected by the inhibitor.

The finding that efflux pump inhibitors did not affect fluconazole MIC values prompted further analysis at the molecular level, specifically examining the expression of genes involved in the efflux system. The results showed that the ATM1 gene was more active in fluconazole-resistant isolates, both when treated and when not treated. The ATM1 gene does not function like an efflux pump, but maintains mitochondrial metabolic stability following damage caused by antifungal drugs. Another transporter gene linked to drug efflux mechanisms is PDR10. 4 weeks of

clotrimazole exposure in sensitive Malassezia furfur strains caused an approximately eightfold increase in MIC. However, after discontinuation of the drug, the MIC value decreased back to near baseline. This change was accompanied by increased PDR10 gene expression during drug exposure and decreased again after discontinuation of therapy. These findings suggest that the decreased sensitivity of Malassezia strains to azoles is dynamic and adaptive, not due to permanent genetic mutations. These findings also strengthen the theory that long-term antifungal exposure may induce physiological adaptations in M. furfur.

Mutations in the CYP51A1 gene, which encodes the azole target enzyme (sterol 14α-demethylase), were found to be associated with increased fluconazole MICs, but were not shown to independently increase ketoconazole MICs. Instead, the increased ketoconazole MICs were more likely related to increased efflux pump activity, primarily through overexpression of the PDR10 gene, than to structural mutations in the target gene.

Overall, the results of this study reinforce the concept that decreased azole sensitivity in Malassezia spp. involves two main, complementary mechanisms: biofilm formation, which creates a physical and physiological barrier to drug penetration; mutations in azole target enzymes; and increased efflux pump activity mediated by ATM1 gene expression, which reduces intracellular antifungal concentrations.

Based on the findings of various in vitro studies, antifungal sensitivity in Malassezia spp. exhibits species-specific variations, drug type, and clinical use. Although azoles remain the mainstay of therapy, fluconazole exhibits the least consistent activity and is strongly influenced by biofilm formation and intrinsic molecular mechanisms. In contrast, ketoconazole and itraconazole exhibit more stable activity, although prior exposure to antifungals or corticosteroids may select for more tolerant fungal subpopulations.

The fungal defense mechanism against fluconazole involves changes in sterol composition, which alter the lipid structure of the cell membrane, rendering it resistant to the drug's effects. It also involves increasing the efflux pump (ABC) by increasing the number of special "pumps" to remove drugs from the cell and increasing the efflux pump (MFS) by adding another type of "pump" to remove the remaining drug from the cell. Another mechanism is through decreasing drug affinity by making it difficult for drug molecules to bind to their target enzyme (ERG11). Overproduction of the target enzyme (ERG11) also plays a role so that there are not enough drug molecules to block everything. Mutations in the ERG11 gene can also change the physical shape of the target enzyme so that the drug cannot recognize it at all (Whaley et al., 2017).

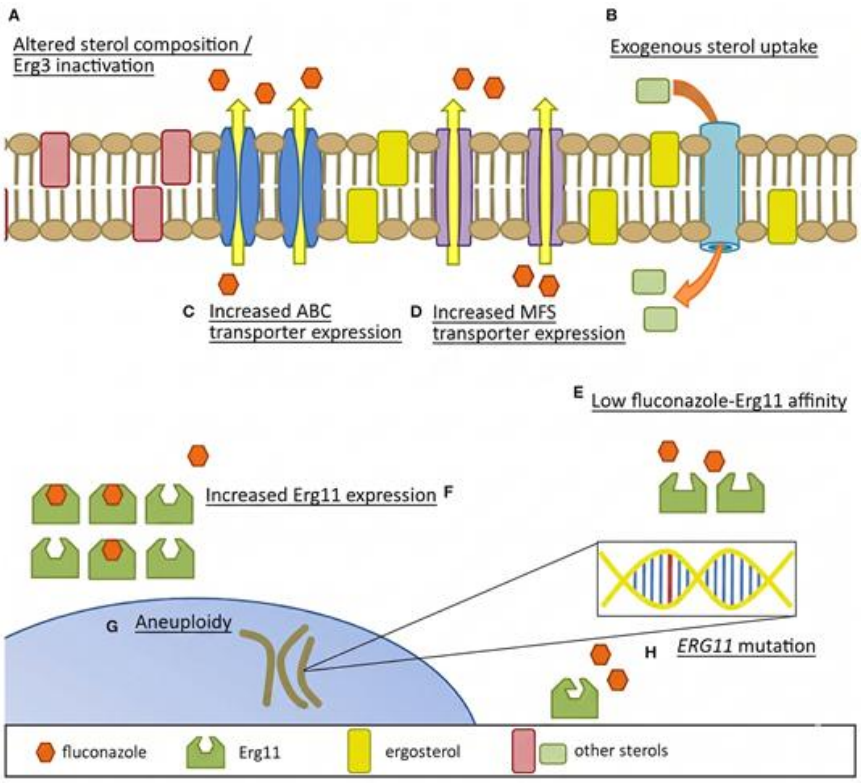

Figure 7. Fluconazole Resistance Mechanism in Fungi

Long-term use of antifungals, particularly topical azoles, causes more sensitive cells to be eliminated earlier, while cells capable of forming biofilms persist and become dominant. This selection process contributes to the emergence of more drug-tolerant fungal populations, reflected in higher MIC values in biofilm cells compared to planktonic cells. Biofilms are not a sign of "normal existence," but rather a sign of "adaptation to stress" (De Pessemier et al., 2025). The main challenges in the development of fungal drug resistance issues against Malassezia are:

a. The lack of a standard CLSI method specifically for Malassezia, so MIC interpretation still refers to Candida spp.
b. The molecular mechanisms of resistance are not fully understood, particularly regarding biofilm formation, expression of efflux pumps, and regulation of transporter genes such as ATM1.

4. Latest Strategies for Resistance Prevention

The most fundamental strategy for preventing antifungal resistance is the routine implementation of antifungal susceptibility testing, especially in cases of recurrent, refractory, or long-term systemic Malassezia infections. Research over the past decade has emphasized the wide variation in MIC values between Malassezia species and strains, suggesting that empirical therapy without susceptibility testing has the potential to encourage the selection of more drug-tolerant fungal strains. Therefore, the use of MIC testing plays a role not only in selecting effective drugs but also as a preventative measure against the selection of resistant strains.

The concept of antifungal stewardship, which encompasses rational drug use based on clear clinical indications, selection of agents with an appropriate spectrum,

limitation of therapy duration, and regular evaluation of clinical response and recurrence, has gained increasing attention in the context of superficial fungal infections in humans, including those involving the genus Malassezia (Ray et al., 2023). In dermatological practice, topical and systemic azoles are the first-line therapy for various Malassezia-associated dermatoses, such as pityriasis versicolor, seborrheic dermatitis, and folliculitis. Therefore, repeated and long-term use is often unavoidable, especially when the disease is chronic and relapsing.

Numerous clinical studies and systematic reviews over the past decade have shown that prolonged exposure to azoles, especially without confirmation of the mycological diagnosis or evaluation of the therapeutic response, can lead to the emergence of more drug-tolerant strains. This contributes to the emergence of isolates with reduced azole susceptibility, reflected in increased minimum inhibitory concentration (MIC) values for species such as Malassezia furfur and Malassezia globosa. This phenomenon becomes even more relevant in patients with chronic diseases, immunocompromised patients, or those requiring long-term maintenance therapy, resulting in frequent recurrent antifungal use. Reduced Malassezia susceptibility to azoles is associated with specific molecular mechanisms, such as mutations in the ERG11/CYP51 gene and increased efflux pump activity, which can be triggered by uncontrolled azole use. Therefore, antifungal stewardship in the context of Malassezia in humans focuses on optimizing therapeutic strategies, including the selection of alternative non-azole agents, appropriate therapy intervals, and consideration of antifungal susceptibility testing in refractory or recurrent cases.

In the last decade, research has also focused on the use of non-azole agents as part of resistance prevention strategies. Combinations with natural products have been reported to have antifungal activity against Malassezia, including strains with reduced azole sensitivity (Deegan et al., 2019). This approach is considered to reduce dependence on azole monotherapy. Combining azoles with adjuvant compounds (drug synergy) can lower azole MICs, broaden the spectrum of activity, and potentially reduce the emergence of drug-resistant Malassezia strains. The synergy of chemical drugs and natural products is a promising approach to preventing the development of resistance (Kane & Carter, 2022).

Table 1. Antifungal Resistance Prevention Strategies in Malassezia spp. Based on Literature

No	Strategy	Explanation	Scientific Basis
1	*Antifungal stewardship*	Rational use of antifungals (indications, dosage, correct duration)	Repeated use of azoles increases the selection of strains with low sensitivity.
2	Antimicrobial sensitivity testing (MIC)-based therapy	Drug selection based on MIC test results	Variation in MIC values between species affects therapeutic response.

3	Restrictions on long-term azole use	Avoid repeated use without clear indications	Increased MICs, especially for fluconazole and ketoconazole, were reported in in vitro studies.
4	Combination therapy	Combination of antifungal with anti-inflammatory agents	A combination approach can increase the effectiveness of therapy and reduce the risk of resistance.
5	Use of topical therapy as first line	Prioritize local therapy in mild–moderate cases	Topical therapy is effective and reduces systemic exposure.
6	Identification of Malassezia species	Species determination due to differences in sensitivity	The sensitivity profiles differ in M. furfur, M. globosa, and M. sympodialis.
7	Monitoring therapy response	Evaluation of therapy success and detection of failure	High MIC values correlate with the likelihood of therapy failure.
8	Patient education	Compliance education and relapse prevention	High recurrence in Malassezia-related diseases (up to 80%).
9	Development of new agents/adjuvants	Exploration of alternative therapies with antifungal and anti-inflammatory effects	The limitations of current therapies are driving the development of new strategies.
10	Addressing predisposing factors	Control risk factors such as temperature, humidity, and immunity.	Host factors influence Malassezia colonization and recurrence.

Table 2. Research results of natural ingredients that have potential as anti-Malassezia

No	Natural Ingredients	Test Design	Results (MIC)	Working Mechanism
1	EGCG (Epigallocatechin gallate) – Camellia sinensis	In vitro (broth microdilution, biofilm assay)	MIC ± 1.2 ± 1.1 mg/mL (depending on strain)	Membrane disruption, ergosterol inhibition, anti-biofilm

2	Zingiber officinale, Alpinia galanga, Curcuma longa, Zingiber cassumunar	In vitro (broth microdilution)	MIC 0.04–0.08 mg/mL	Phenolic compounds and essential oils → membrane disruption and cell metabolism
3	Propolis (bee product)	In vitro (agar diffusion + microdilution)	MIC 1.22 mg/mL (potent against M. globosa)	Lipase and cell wall inhibition
4	Neem (Azadirachta indica)	In vitro	MIC 14mm compared to ketoconazole 17mm	Antimicrobials through metabolic disorders
5	Nyctanthes arbor-tristis	In vitro	MIC M. globosa 1.05 µg/µl, M. restricta MIC 1.47 µg/µl	Inhibition of cell growth and metabolism
6	Citrullus colocynthis	In vitro (broth microdilution)	MIC equivalent to ketoconazole 0.7 ± 0.5 µg/mL	Cell membrane disruption
7	Bajakah (Uncaria acida)	In vivo (mouse model)	Optimal effectiveness at 75% extract	Flavonoids and saponins → antifungal + anti-inflammatory
8	Cocoa Leaf Extract (Theobroma cacao)	In vitro (macrodilution)	The 50% concentration was determined as the MIC because it is the lowest concentration that shows a decrease in the optical density (OD) value, which indicates inhibition of Malassezia furfur growth.	Flavonoids, tannins → disruption of cell membranes and proteins

Various natural products, particularly phytochemical compounds, exhibit significant antifungal activity against Malassezia spp. through mechanisms involving cell membrane disruption, biofilm inhibition, and modulation of virulence factors.

These findings demonstrate the potential of natural products as alternative or adjuvant therapies to address decreased antifungal sensitivity. Numerous in vitro studies have shown that EGCG and other catechins have inhibitory activity against skin fungi (including Malassezia in some studies). The mechanisms described include damage to the fungal cell wall/membrane, disruption of ergosterol synthesis, and induction of fungal cell death. This has been reported in in vitro studies against dermatophytes, Candida, and specific studies of Malassezia isolates. In vitro studies have shown that the combination of catechin (EGCG) with azoles or miconazole can lower the MIC of antifungal drugs, suggesting a synergistic effect or potential adjuvant activity that could theoretically allow for azole dose reduction and reduce the dominance of fungal subpopulations with reduced susceptibility (de Araújo Medeiros et al., 2022).

The strategy of preventing Malassezia resistance through combination therapy (azoles with natural products or adjuvants) is supported by strong in vitro evidence and has an effective synergistic mechanism of action to inhibit the development of drug-resistant fungal strains. However, clinical evidence in humans is still limited, so clinical application requires controlled clinical trials, safety formulation studies, and ongoing resistance monitoring.

E. CONCLUSION

Decreased antifungal sensitivity in Malassezia spp. is an increasingly reported phenomenon and has direct implications for the success of clinical therapy. Increasing MIC values, particularly for fluconazole, indicate a trend toward decreased sensitivity, which can lead to therapy failure and disease relapse. In contrast, posaconazole and ketoconazole still demonstrate relatively good activity, although they are not without the potential for decreased sensitivity. The mechanisms of decreased sensitivity involve changes in drug targets, increased efflux pumps, and biofilm formation. Therefore, a more rational approach to antifungal use is needed, including the application of antifungal stewardship, sensitivity testing-based therapy selection, and the development of combination therapy strategies, such as those using natural products. These efforts are expected to suppress the development of resistance and increase the effectiveness of therapy for skin diseases associated with Malassezia spp.

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