Investigating Potentials of Actinomycete-derived Metabolites against Drug-resistant Fungal Infections: A Comprehensive Review of The Filamentous Bacterial Efficacy in Oropharyngeal Candidiasis

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Abstract

The increasing prevalence of drug-resistant fungal infections, particularly oropharyngeal candidiasis, represents a major global health concern, especially in immunocompromised populations. This review critically investigated the potential of actinomycete-derived secondary metabolites as alternative or adjunct therapeutic agents against antifungal-resistant fungal pathogens, with a primary focus on *Candida albicans*. Actinomycetes are well recognized for their capacity to produce structurally diverse bioactive compounds, including polyketides and non-ribosomal peptides, many of which demonstrate potent antifungal activity and novel mechanisms of action capable of bypassing established resistance pathways.

This review has summarized current knowledge on the antifungal efficacy of actinomycete metabolites, their mechanisms of action and reported synergistic interactions with conventional antifungal agents. In addition, key limitations delaying clinical translation—such as cultivation challenges, toxicity concerns, formulation barriers, pharmacokinetic constraints and regulatory considerations—have critically been discussed. By integrating microbiological, pharmacological and translational perspectives, this review has highlighted promise and current gaps in development of actinomycete-derived antifungal agents. Addressing these challenges through advanced genomic tools, rational screening strategies and translational research is essential for advancing actinomycetes toward clinical use in oropharyngeal candidiasis and other drug-resistant fungal infections.

Keywords: Actinomycetes, antifungal agents, oropharyngeal candidiasis

1. Context

Fungal infections have emerged as a significant public health challenge, particularly in immunocompromised individuals, including patients with human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS), malignancies undergoing chemotherapy and organ transplant recipients [1]. The increasing incidence of these infections is driven by multiple factors, such as prolonged antifungal exposure, widespread use of broad-spectrum antibiotics, increasing numbers of immunocompromised patients and environmental changes that favor fungal adaptation and persistence [2]. Among fungal pathogens, *Candida albicans* is still one of the most prevalent opportunistic species, largely due to its significant ability to develop resistance through target enzyme mutations, efflux pump overexpression and biofilm formation; thereby, decreasing the effectiveness of conventional antifungal agents, including azoles, echinocandins and polyenes. The increasing threat of antifungal resistance has formally been recognized by the World Health Organization (WHO) as a critically global health priority [1].

Oropharyngeal candidiasis (OPC) is one of the most common clinical manifestations of *Candida* infection and frequently serves as an indicator of immune dysfunction, particularly in individuals with advanced HIV infection. Clinically, OPC is characterized by white or erythematous lesions affecting the oral mucosa and oropharynx, often accompanied by pain, dysphagia and compromised quality of life. The condition is especially prevalent in patients receiving corticosteroids, broad-spectrum antibiotics or chemotherapy, which disrupt normal oral microbiota and facilitate fungal overgrowth. Recurrent or chronic OPC can progress to more severe complications, including esophagitis and systemic dissemination, particularly in elderly patients, individuals with diabetes and those with prolonged immunosuppression. These clinical challenges underscore the urgent need for novel and further effective antifungal strategies capable of overcoming drug resistance [3].

Actinomycetes, a diverse group of Gram-positive filamentous bacteria, represent a promising reservoir for antifungal drug discovery due to their exceptional ability to produce structurally and functionally diverse secondary metabolites. These microorganisms occupy a wide range of ecological niches, including terrestrial, marine and extreme environments, contributing to their metabolic diversity. Historically, actinomycetes—particularly members of the *Streptomyces* genus—have been sources of numerous clinically important antimicrobial agents, such as

streptomycin, tetracycline and erythromycin. Advances in genomic, metagenomic and genome-mining technologies have revealed the presence of numerous cryptic biosynthetic gene clusters within actinomycete genomes, highlighting their untapped potential for antifungal compound discovery [4,5].

This review has critically investigated current research on actinomycete-derived metabolites as potential therapeutic agents against drug-resistant fungal infections, with a specific focus on OPC. The review has synthesized available evidence on antifungal efficacy, mechanisms of action and synergistic interactions with available antifungal drugs while addressing key translational challenges, including bioavailability, toxicity, formulation and regulatory hurdles. By integrating insights from microbiology, pharmacology and clinical research, this study has aimed to provide a comprehensive framework for advancing actinomycete-based antifungal strategies and identify future research priorities necessary for successful clinical translation.

1.1 Drug Resistance in Fungal Pathogens

1.1.1 Mechanisms of Antifungal Resistance in *Candida albicans*

Antifungal resistance in *C. albicans*, one of the most prevalent opportunistic fungal pathogens, represents a major clinical challenge, particularly in the management of OPC. Resistance to commonly used antifungal classes—including azoles, echinocandins and polyenes—has increasingly been reported in clinical isolates, contributing to treatment failure and recurrent infections [1]. Understanding the molecular and cellular mechanisms underlying this resistance is essential for the development of novel antifungal strategies and effective adjunct therapies.

One of the primary mechanisms of antifungal resistance in *C. albicans* involves alterations in drug target enzymes. Azole antifungals inhibit ergosterol biosynthesis by targeting lanosterol 14-α-demethylase, encoded by the *ERG*11 gene. Point mutations in *ERG*11 can significantly decrease azole-binding affinity, leading to decreased susceptibility. Common substitutions, such as Y132F and G448S, have strongly been associated with fluconazole resistance in clinical isolates. In addition to target mutations, *ERG*11 overexpression—often mediated by transcriptional regulators such as *UPC*2—can enhance resistance by increasing enzyme abundance [6].

Another critical resistance mechanism is the upregulation of efflux pumps, which actively expel antifungal agents from fungal cells. *C. albicans* predominantly uses ATP-binding cassette (ABC)

transporters (e.g., Cdr1p and Cdr2p) and major facilitator superfamily (MFS) transporters (e.g., Mdr1p). Overexpression of these transporters results in decreased intracellular drug accumulation, conferring cross-resistance to multiple antifungal agents. This efflux-mediated resistance is particularly relevant in chronic OPC, where prolonged antifungal exposure promotes adaptive responses [6]. Biofilm formation contributes significantly to antifungal resistance in *C. albicans*. Biofilms formed on mucosal surfaces and medical devices demonstrate enhanced resistance to antifungal agents and host immune responses. Extracellular matrix production and biofilm-associated adaptive responses contribute to the persistence of these infections [7].

Additional resistance mechanisms include alterations in membrane sterol composition, which decrease the binding affinity of polyenes such as amphotericin B and adaptive stress response pathways that enhance fungal survival under antifungal pressure. Collectively, these multifactorial resistance strategies highlight the limitations of current antifungal therapies and emphasize the need for alternative agents with novel mechanisms of action [8]. Actinomycete-derived metabolites are of particular interest in this context, as many demonstrate unique targets or multi-site modes of action that may bypass conventional resistance pathways. Therefore, a clear understanding of resistance mechanisms in *C. albicans* provides a rational foundation for investigating actinomycetes as sources of next-generation antifungal compounds.

Table 1. Major mechanisms of antifungal resistance in *Candida albicans*

Resistance mechanism	**Molecular basis**	**Affected antifungal class**	**Clinical relevance**	**Ref.**
Target enzyme mutation	*ERG*11 point mutations (e.g., Y132F, G448S)	Azoles	Decreased drug binding, treatment failure	[6, 37]
Target overexpression	Upregulation of *ERG*11 via *UPC*2 and *TAC*1 transcriptional activation	Azoles	Increased ergosterol synthesis	[1]
Efflux pump activation	Overexpression of Cdr1/Cdr2 (ABC) and Mdr1 (MFS) transporters	Azoles, echinocandins	Multidrug resistance	[6]
Biofilm formation	ECM barrier, metabolic heterogeneity, biofilm-specific gene expression	All major classes	Chronic, recurrent OPC	[7,28]

Membrane sterol alteration	Changes in ergosterol composition reducing polyene binding	Polyenes	Decreased amphotericin B efficacy	[6]
Stress response adaptation	Activation of oxidative, osmotic and cell-wall stress pathways	Multiple classes	Enhanced fungal survival	[1, 13]

1.1.2 Implications of Drug Resistance on Treatment Options and Patient Outcomes

The increasing prevalence of antifungal resistance in *C. albicans* and other fungal pathogens presents a substantial obstacle to effective clinical management. As resistance mechanisms become more widespread, the therapeutic landscape is progressively constrained, creating complex clinical scenarios that directly affect patient prognosis and healthcare delivery. A thorough understanding of these implications is essential for optimizing treatment strategies and improving outcomes. One of the immediate consequences of antifungal resistance is the restriction of effective therapeutic options. For example, infections caused by azole-resistant *Candida* species may no longer respond to first-line agents such as fluconazole. In such cases, clinicians may need to use alternative antifungal agents, including amphotericin B and echinocandins [1]. These therapeutic adjustments complicate clinical decision-making and may increase the likelihood of suboptimal responses, prolonged recovery and/or persistent infection.

The dynamic evolution of resistance also contributes to more complicated and difficult-to-control infections, particularly in immunocompromised individuals and patients with chronic underlying diseases. Failure of standard regimens may necessitate combination antifungal therapy, which, although potentially effective against resistant isolates, introduces additional risks such as drug-drug interactions and cumulative toxicity [9]. Consequently, intensified monitoring is often necessary, increasing the clinical workload and resource use within healthcare settings. The chronic nature of drug-resistant fungal infections significantly affects patient prognosis. Evidence indicates that resistant infections are associated with increased morbidity and mortality. Multidrug-resistant *C. albicans* can progress to invasive and/or disseminated disease, particularly in immunocompromised patients. Although early diagnosis and targeted therapy are critical determinants of success, presence of resistance decreases therapeutic predictability and diminishes the possibility of rapid clinical resolution [1].

Drug-resistant OPC, in particular, represents a challenging clinical manifestation with broader systemic implications. Recurrent or resistant OPC often requires prolonged or modified antifungal therapy, especially in immunocompromised populations such as individuals living with HIV/AIDS and patients receiving intensive medical treatments. Persistent OPC may complicate clinical management, increase the need of microbiological identification and antifungal susceptibility testing and necessitate alternative therapeutic strategies when conventional agents become less effective. Beyond its direct clinical effects, resistant OPC can increase the burden on healthcare services by requiring repeated clinical assessments, additional diagnostic procedures and further specialized antifungal management. Thus, resistant OPC constitutes not only a therapeutic difficulty but also a meaningful clinical and healthcare-management challenge [10].

In addition to clinical consequences, persistent and drug-resistant fungal infections impose a broader public health burden. Recurrent infections and decreased treatment effectiveness can complicate patient management, particularly because antifungal resistance limits available therapeutic options and may contribute to repeated or prolonged treatment courses. From a broader perspective, the increasing emergence of antifungal resistance increases important public health concerns. Therefore, robust surveillance systems are essential to monitor resistance patterns, improve diagnostic capacity, guide empirical therapy and inform evidence-based treatment and stewardship strategies [1]. Education of healthcare professionals regarding antifungal stewardship principles is equally critical to preserve available therapeutic options and limit emergence of resistant strains.

Overall, antifungal resistance exerts multidimensional effects on therapeutic decision-making, patient outcomes, healthcare costs and public health systems. As resistant fungal infections become increasingly prevalent, clinicians and policymakers must prioritize early detection, individualized treatment strategies, stewardship initiatives and preventive measures. Coordinated efforts at the patient and health system levels are imperative to lessen the increasing threat posed by antifungal resistance and to safeguard further treatment efficacy.

1.2 Current Antifungal Therapies and Their Limitations

The management of fungal infections, particularly those caused by opportunistic pathogens such as *C. albicans*, relies heavily on antifungal therapies. Over the years, several classes of antifungal agents have been developed, each with distinct mechanisms of action, advantages and limitations.

Understanding these therapies and their limitations is critical for clinicians to optimize treatment outcomes and address the challenges posed by antifungal resistance.

1.2.1 Azoles

Azoles, including fluconazole, itraconazole and voriconazole, are some of the most commonly used antifungal agents. They act by inhibiting the enzyme lanosterol 14-α-demethylase, which is critical for ergosterol synthesis, an essential component of fungal cell membranes. Azoles are effective against various *Candida* spp., making them first-line treatments for several superficial and systemic infections. However, their use is limited by several factors. Prolonged use of azoles can lead to the development of resistance through mutations in the *ERG*11 gene and the upregulation of efflux pumps [6]. Resistance mechanisms severely limit the effectiveness of azoles, particularly in patients with recurrent infections, necessitating alternative therapies.

1.2.2 Echinocandins

Echinocandins such as caspofungin, micafungin and anidulafungin, represent a newer class of antifungals that target the fungal cell wall by inhibiting the synthesis of β-(1,3)-D-glucan, a critical component of the cell wall in *Candida* spp. [11]. This mechanism of action not only provides efficacy against azole-resistant strains but also confers a favorable safety profile. However, echinocandins demonstrate important limitations. Their activity is fungicidal against *Candida* species but largely fungistatic against filamentous fungi such as *Aspergillus*, which may contribute to incomplete pathogen clearance in certain settings. Moreover, their efficacy is partly dependent on host immune function, increasing concerns regarding optimal use in severely immunocompromised patients, where combination or alternative antifungal strategies may be necessary. Moreover, echinocandins are not effective against all species of *Candida*, especially *C. parapsilosis*, which can present clinical challenges in managing infections [12].

1.2.3 Polyenes

Polyene antifungals, with amphotericin B as the most well-known polyene, interfere with ergosterol in the fungal cell membrane, leading to pore formation and cell death. Amphotericin B is often used in severe systemic infections due to its broad spectrum of action, including activity against resistant fungi [13]. However, clinical use of amphotericin B is complicated by significant limitations, including severe nephrotoxicity and infusion-linked reactions, which can restrict its

use particularly in at-risk populations [14]. Liposomal formulations have been developed to decrease toxicity while maintaining efficacy. However, these formulations are significantly expensive, posing a cost barrier in healthcare settings.

1.2.4 Nucleotide Analogs

Nucleotide analogs such as flucytosine are occasionally used in combination with other antifungals to enhance treatment efficacy, especially in cases of cryptococcal meningitis. Flucytosine acts by inhibiting DNA and RNA synthesis. However, its clinical use is limited due to the rapid development of resistance when used as a monotherapy, necessitating combination therapy with agents such as amphotericin B to prevent resistance [1]. Additionally, flucytosine has a narrow therapeutic window, needing careful monitoring of drug levels to avoid potential toxicity.

1.2.5 Novel and Investigational Agents

In response to the increasing problem of antifungal resistance, there has been an increased focus on developing novel antifungal agents and investigating alternative therapeutic strategies. Agents such as rezafungin, a novel echinocandin, are clinically assessed, showing promising results against resistant strains of *Candida* [15]. Other investigational compounds are focusing on unique mechanisms, including those targeting fungal biofilms and novel signaling pathways. While these agents offer hope, their long-term safety and effectiveness still need extensive clinical validation.

2. Data Acquisition

Data acquisition was carried out using online databases, including Google Scholar, PubMed and Elsevier.

3. Results

3.1 Actinomycetes: Biological Characteristics, Biosynthetic Capacity and Relevance to Antifungal Drug Discovery

Actinomycetes are Gram-positive filamentous bacteria, belonging predominantly to the phylum Actinobacteria. Of them, members of the *Streptomyces* genus represent the most extensively studied group due to their significant capacity to produce structurally diverse secondary metabolites [16]. Unlike most bacteria, actinomycetes demonstrate a filamentous growth pattern resembling fungi, including substrate and aerial mycelia formation, with complex morphological

differentiation. This developmental complexity is closely linked to secondary metabolite biosynthesis, which is often triggered during the transition from vegetative growth to sporulation [17].

3.2 Ecological Adaptation and Chemical Diversity

The extraordinary biosynthetic diversity of actinomycetes is suggested as an ecological adaptation. These microorganisms inhabit competitive environments such as soil, marine sediments, plant endospheres and extreme ecological niches, where chemical warfare provides a survival advantage [17]. Therefore, actinomycetes evolved the ability to synthesize a vast array of bioactive molecules, including polyketides, non-ribosomal peptides, aminoglycosides, macrolides and terpenoids. Importantly, genome-sequencing studies have revealed that single *Streptomyces* genome may contain 20–40 biosynthetic gene clusters (BGCs), many of which are silent under laboratory conditions [17]. This indicates that the known metabolites likely represent only a fraction of their biosynthetic potential. Such cryptic gene clusters are increasingly viewed as a reservoir for next-generation antifungal compounds, particularly relevant in the context of multidrug-resistant *Candida* spp.

3.3 Historical Contribution and Translational Relevance

Actinomycetes have historically transformed antimicrobial therapy. Important discoveries such as streptomycin, tetracycline, erythromycin and vancomycin are originated from actinomycete species [18]. In antifungal therapy specifically, polyenes such as nystatin and amphotericin B were isolated from *S. noursei* and *S. nodosus*, respectively and remain clinically indispensable decades after their discovery [19]. However, despite this success, antifungal drug development has lagged behind antibacterial research. Only a limited number of antifungal classes are currently available and resistance is increasing in *C. albicans*, *C. auris* and other opportunistic pathogens. Given their proven biosynthetic versatility, actinomycetes represent one of the most rational microbial sources for expanding the antifungal pipeline.

3.4 Biosynthetic Machinery and Mechanistic Implications

The antifungal capacity of actinomycetes is linked to their diverse biosynthetic systems, which generate structurally complex metabolites with potential activity against multiple fungal targets [17, 18]. This multi-target potential is particularly relevant given the adaptive mechanisms

described in Section 2 (e.g., efflux pumps, *ERG*11 mutations, biofilm-mediated resistance). Compounds with unconventional targets may decrease cross-resistance with available azoles or echinocandins. Nevertheless, it is critical to acknowledge that not all actinomycete-derived metabolites possess favorable pharmacological profiles. Issues such as cytotoxicity, poor solubility and limited bioavailability have historically limited translational success [11, 20]. Therefore, while biosynthetic diversity is vast, systematic prioritization based on antifungal selectivity and therapeutic index remains essential.

3.5 Critical Gaps and Strategic Directions

3.5.1 Resistance Evolution Studies

Limited research has systematically investigated how fungal pathogens adapt to actinomycete-derived compounds over time. Given the well-characterized capacity of *Candida* spp. to develop resistance through efflux pump overexpression, target mutations and stress-response rewiring [21], understanding resistance dynamics to novel actinomycete metabolites is essential prior to clinical translation.

3.5.2 Pharmacodynamic Modeling in OPC Context

Considering the localized mucosal nature of OPC, topical antifungal approaches may help decrease systemic exposure and associated toxicity. Nevertheless, pharmacokinetic and pharmacodynamic considerations specific to OPC have received comparatively limited attention [21].

3.6 Antifungal Metabolites from Actinomycetes

Actinomycetes represent one of the richest sources of bioactive secondary metabolites in antimicrobial drug discovery. From the structurally diverse compounds produced by these filamentous bacteria, several classes demonstrate antifungal characteristics through distinct mechanisms of action. Importantly, not all metabolites traditionally classified within well-known antibiotic groups possess intrinsic antifungal activity; therefore, clear mechanistic distinction is essential.

3.7 Types of Antifungal Compounds Produced by Actinomycetes

3.7.1 Macrolides

Although classical antibacterial macrolides are not considered primary antifungal agents, polyene macrolides constitute a distinct subgroup with intrinsic antifungal activity. Representative compounds include amphotericin B and nystatin, which bind ergosterol within fungal membranes, leading to pore formation and cell death [13]. Their mechanism is membrane-targeted rather than ribosomal. Despite nephrotoxicity concerns, polyene macrolides remain essential in the management of invasive candidiasis and systemic mycoses. Advances in biosynthetic gene cluster mining and semi-synthetic modification strategies continue to generate novel macrolide derivatives with improved safety and enhanced antifungal selectivity [22].

3.7.2 Peptides and Lipopeptides

Actinomycetes produce diverse ribosomal and non-ribosomal peptides with antifungal activity. Lipopeptides frequently exert antifungal effects through membrane interaction or disruption of intracellular processes. These mechanisms are particularly relevant against *Candida* strains. In addition to membrane disruption, certain peptide metabolites inhibit fungal growth. Continued investigation of marine actinomycetes has expanded the diversity of antifungal peptides identified in recent years [3, 23].

3.7.3 Polyketides

Polyketides represent one of the most structurally complex and pharmacologically significant classes of actinomycete metabolites. The most clinically significant antifungal polyketide is amphotericin B, derived from *S. nodosus*. Its high affinity for ergosterol disrupts membrane integrity and induces membrane destabilization and downstream oxidative stress responses [24]. Although toxicity limits its use, liposomal and lipid-complex formulations have improved therapeutic indices. Beyond classical polyene compounds, genomic mining of actinomycetes has revealed numerous cryptic biosynthetic gene clusters encoding potentially novel polyketides with antifungal activity [25]. Structural diversification within this class enables targeting of multiple fungal pathways, decreasing the likelihood of resistance development compared with single-target azoles.

3.7.4 Other Novel Secondary Metabolites

Actinomycetes produce alkaloids, terpenoids, isocoumarins and anthracycline-like compounds with reported antifungal activity. Marine-derived metabolites, in particular, have shown promising

bioactivity against *Candida* species [26]. The discovery of such compounds underscores the importance of continued bioprospecting and activation of silent biosynthetic pathways through genomic engineering approaches.

3.8 Mechanisms of Action of Actinomycete Metabolites against Fungi

Actinomycete-derived antifungal metabolites use various mechanisms of action that disrupt key cellular processes in fungal pathogens. Understanding these mechanisms is vital for optimizing therapeutic strategies and overcoming resistance. One primary mechanism involves the disruption of fungal cell membranes. Several antifungal compounds, such as amphotericin B and nystatin, interact with ergosterol, a vital component of fungal membranes, leading to pore formation and increased permeability, which ultimately results in cell death [27]. Disruption of membrane integrity compromises fungal cell viability and represents a major antifungal target.

Another critical mechanism of action is the inhibition of fungal cell wall synthesis. Some metabolites disrupt critical enzymes involved in chitin or β-glucan synthesis, thereby compromising the structural integrity of the fungal cell wall. For example, echinocandins, semisynthetic lipopeptides derived from fungal metabolites, inhibit 1,3-β-D-glucan synthase, a key enzyme in the biosynthesis of fungal cell walls, effectively decreasing cell wall integrity and leading to cell lysis [27]. In contrast to antibacterial macrolides such as erythromycin—which bind to the bacterial 50S ribosomal subunit and do not directly inhibit fungal cytoplasmic protein synthesis [27]—certain actinomycete-derived metabolites can interfere with intracellular fungal processes through alternative mechanisms. Some non-ribosomal peptides and secondary metabolites modulate stress response pathways, disrupt mitochondrial function or interfere with signaling cascades essential for fungal survival and biofilm persistence [21, 28]. These indirect or multi-target effects may enhance antifungal efficacy and decrease the likelihood of resistance development.

Induction of oxidative stress is another antifungal mechanism used by actinomycetes. Certain metabolites can generate reactive oxygen species (ROS), leading to oxidative damage [23]. This oxidative stress often results in cell damage through lipid peroxidation, resulting in apoptosis or necrosis. In addition, the inhibition of nucleic acid synthesis has been reported for actinomycete metabolites. For example, actinomycin D inhibits RNA elongation by RNA polymerase, thereby blocking transcription. This ultimately leads to the termination of protein synthesis and hence cell

growth [23]. Such multifaceted mechanisms highlight the potential for designing combination therapies targeting multiple pathways to combat resistance and enhance antifungal efficacy. Overall, understanding the diverse mechanisms of action of antifungal metabolites from actinomycetes not only informs therapeutic strategies but also helps in the search for novel compounds that can effectively target resistant fungal pathogens.

3.9 Case Studies of significant Actinomycete-Derived Metabolites with Antifungal Activity

Actinomycetes have yielded numerous clinically important metabolites with potent antimicrobial and antifungal activities. In this review, several case studies have been reported that exemplify their antifungal characteristics and mechanisms of action. A well-studied example is nystatin, a polyene macrolide antifungal originally derived from *S. noursei*. Nystatin acts by interacting with ergosterol in fungal membranes, leading to membrane disruption, pore formation, leakage of intracellular components and eventual fungal cell death. In contrast, antibacterial macrolides such as erythromycin, originally produced by *Saccharopolyspora erythraea*, are primarily recognized for antibacterial activity rather than direct antifungal action [22]. It was one of the first antifungal agents discovered and is used for treating superficial *Candida* infections. The effectiveness of nystatin against azole-resistant strains has renewed interest in its clinical use, particularly in immunocompromised populations.

Amphotericin B, another critical actinomycete derivative sourced from *S. nodosus*, has been used in managing systemic fungal infections. Its fungicidal activity against various pathogens, including *Aspergillus* and *Cryptococcus* spp., is well documented with its capacity to bind to ergosterol disrupting cellular membranes [19]. Another important direction is the discovery of novel antifungal metabolites from microbial sources, including *Streptomyces* spp. Natural polyketides and related metabolites have shown diverse antifungal activities and mechanisms of action, highlighting the importance of continued bioprospecting and biosynthetic-pathway investigation for identifying actinomycete strains with untapped therapeutic potential [29]. Such findings suggest that systematic investigations into understudied actinomycetes can lead to significant advancements in antifungal therapy. Indeed, several clinically relevant antifungals such as natamycin (a polyene macrolide) derived from *S. chattanoogensis* highlight the continued importance of actinomycetes as a prolific source of bioactive compounds. These metabolites

demonstrate the structural diversity and therapeutic potential of actinomycete-derived natural products, underscoring the need of continued investigation of these organisms [26, 30].

3.10 Use of Actinomycetes in Treating Oropharyngeal Candidiasis

3.10.1 Overview of Current Treatment Strategies for Oropharyngeal Candidiasis

The OPC, predominantly caused by *C. albicans*, is a common opportunistic infection affecting immunocompromised individuals, including those with HIV/AIDS that receive chemotherapy and/or immunosuppressive therapies [31]. The clinical management of OPC typically involves the use of antifungal agents tailored to the severity and resistance patterns of the infections. While mild cases of OPC may be managed effectively with topical antifungals such as clotrimazole or miconazole, further severe cases often necessitate systemic therapies, including fluconazole and other azoles [32]. Current treatment strategies primarily rely on azole antifungals. However, increasing reports of resistance in *Candida* spp., particularly *C. albicans* and non-*albicans* species, have increased significant concerns regarding the long-term effectiveness of standard treatments The emergence of resistance is often attributed to the overuse of antifungal agents, which leads to increased treatment failures [33]. Regarding these challenges, there is an urgent need of innovative therapeutic options, including the investigation of actinomycete-derived metabolites with antifungal characteristics.

The current clinical guidelines emphasize early diagnosis and prompt initiation of appropriate antifungal therapy to lessen complications and improve patient outcomes. A different approach is often used, where mild infections are treated with topical agents while systemic azoles are reserved for further complicated cases [32]. However, limitations of available antifungals, coupled with the potential for adverse effects, necessitate the ongoing search for alternative treatments. Therefore, actinomycete metabolites may play a critical role as potential adjuncts or alternatives in OPC management. In addition to pharmacological interventions, preventive measures to control OPC include maintaining good oral hygiene, especially for at-risk populations. Food modification and dietary strategies aimed at decreasing sugar intake may help in minimizing the risk of candidiasis development [32]. Despite the progress in understanding OPC, the clinical view is evolving and novel therapeutic strategies, particularly those with natural products from actinomycetes, are essential to enhance the current arsenal against this pervasive fungal infection.

3.10.2 Preclinical and Clinical Studies Investigating Actinomycete Metabolites against Oropharyngeal Candidiasis

Preclinical studies investigating the antifungal potential of actinomycete metabolites have yielded promising results, showing their efficacy against *Candida* spp. and specifically addressing their use in OPC. Studies have demonstrated that several actinomycete-derived compounds include significant inhibitory effects on the growth of *C. albicans*; thus, warranting clinical examination. For example, extracts from *Streptomyces* spp. have shown dose-dependent antifungal activity *in vitro*, which has led to further investigations regarding their mechanisms of action [34]. An important study demonstrated the potent anti-*Candida* activity of partially purified secondary metabolites from *S. chrestomyceticus* strain ADP4. These metabolites effectively inhibited biofilm formation and significantly decreased the expression of key virulence factors (e.g. phospholipase and proteinase) in *C. albicans*. The multi-target effects on biofilm architecture and virulence suggest a promising potential for enhancing the efficacy of conventional azoles, such as fluconazole, particularly against biofilm-associated resistant infections [35]. These findings are mostly interesting since biofilm-associated *C. albicans* infections are significantly highly resistant to conventional antifungal therapies, increasing the clinical urgency to identify novel treatment modalities.

Additionally, several studies used screening methods to isolate novel actinomycete strains and assess their metabolites against OPC pathogens. A systematic investigation of soil-derived actinomycetes led to the identification of several strains capable of producing potent antifungal compounds [36]. Especially, these compounds demonstrated minimal inhibitory concentrations (MICs) similar to those of standard antifungal agents, highlighting the potential of these metabolites as a source of new therapeutic agents. Clinical trials investigating the safety and efficacy of these actinomycete-derived metabolites in OPC settings are critical for translation of promising preclinical findings into practical therapeutic uses. While the research has mostly been *in vitro*, further steps involve rigorous clinical assessments to ascertain the effectiveness of these compounds in human subjects. Emerging studies aim to investigate novel formulations incorporating the antifungal characteristics of actinomycete metabolites to deliver improved therapeutic coverage in OPC. Furthermore, there is inherent potential in combining actinomycete-derived antifungal agents with current antifungal therapies, which can potentiate their efficacy and

address resistance concerns. As clinicians and researchers enhance the current therapeutic strategies, it is essential to consider the implications of actinomycete-derived compounds in real-world clinical scenarios [37].

3.10.3 Synergistic Potential of Actinomycetes with current Antifungal Treatments

The emergence of multidrug-resistant strains of *Candida* necessitates innovative strategies that include combination therapies to enhance antifungal efficacy and overcome resistance mechanisms. Recent studies have highlighted the synergistic potential of actinomycete metabolites in combination with the current antifungal agents in treating OPC. This dual approach not only maximizes the therapeutic outcomes but also may decrease the likelihood of developing resistant fungal strains [38]. A recent study demonstrated that metabolites derived from *Streptomyces* spp. demonstrate significant antifungal activity against fluconazole-resistant *C. albicans*, with evidence suggesting enhanced efficacy when combined with azole therapy, supporting the potential for synergistic interactions between actinomycete-derived compounds and conventional antifungals [37].

Additionally, non-ribosomal peptides, particularly lipopeptides derived from actinomycetes, have shown significant potential for synergistic interactions with available antifungal agents. Lipopeptides from *Streptomyces* spp. demonstrate strong membrane-disrupting and antibiofilm characteristics that can enhance the efficacy of azoles and echinocandins against resistant *Candida* strains [39, 40]. These combinations not only improve drug penetration into biofilms but also target multiple cellular pathways, helping overcome resistance mechanisms and potentially leading to better clinical outcomes in OPC and other invasive candidal infections. Several studies have demonstrated that lipopeptides demonstrate potent antibiofilm activity against *Candida* spp. and can enhance the efficacy of conventional antifungal agents, particularly fluconazole, through membrane disruption and biofilm inhibition mechanisms [40]. The combination therapy significantly inhibited biofilm formation and decreased cell viability, highlighting the capacity for actinomycete-derived compounds to enhance the clinical effectiveness of available therapies.

Table 2. Reported synergistic combinations of actinomycete-derived metabolites with conventional antifungal agents against *Candida* spp.

Actinomycete-derived metabolite	**Producing organism**	**Partner antifungal**	**Proposed mechanism of synergy**	**Target model**	**Reported outcome**	**Ref.**
Erythromycin (adjunct macrolide)	*Saccharopolyspora erythraea*	Fluconazole	Modulation of fungal physiology; potential enhancement of antifungal susceptibility and biofilm interference	*Candida albicans*	Altered susceptibility and enhanced antifungal effect in combination context	[22]
Nystatin (polyene macrolide)	*Streptomyces noursei*	Azoles	Ergosterol binding and inhibition of ergosterol synthesis → membrane destabilization	*Candida albicans*	Enhanced antifungal activity against resistant strains	[22]
Amphotericin B/lipid formulations	*Streptomyces nodosus*	Azoles or combination therapy	Membrane pore formation and complementary intracellular stress	*Candida* spp. including resistant isolates	Broad-spectrum fungicidal activity; improved efficacy in resistant infections	[19, 24]
Partially purified secondary metabolites	*Streptomyces chrestomyceticus* strain ADP4	Fluconazole	Inhibition of biofilm formation, reduction of virulence enzymes (phospholipase, proteinase)	*Candida albicans* biofilm	Strong dose-dependent inhibition of biofilm formation and pre-formed biofilms	[35]
Crude actinomycete metabolites	*Streptomyces* spp.	Fluconazole	Multi-target activity: membrane disruption, oxidative stress,	Clinical *Candida* isolates	Dose-dependent inhibition; synergistic interaction with azoles	[34, 36]

			metabolic interference			
Lipopeptides/secondary metabolites	*Streptomyces* spp.	Conventional antifungals (azoles/echinocandins)	Biofilm disruption and membrane interaction → improved drug penetration	Biofilm-forming *Candida* spp.	Decreased biofilm viability; enhanced antifungal effect	[40]

To provide a structured overview of the currently available evidence, the principal synergistic combinations of actinomycete-derived metabolites with conventional antifungal agents are summarized in Table 2. Most reported interactions involve azoles, echinocandins and polyenes, targeting complementary pathways such as ergosterol biosynthesis, membrane permeability, biofilm formation and lipid metabolism. Across studies, synergy has generally been reflected by decreased MIC values, enhanced biofilm inhibition or partial restoration of antifungal susceptibility in resistant isolates. Although most data are preclinically *in vitro*, the consistency of mechanistic convergence supports further translational investigation using standardized checkerboard assays, time–kill analyses and *in vivo* OPC models.

3.11 Translational Challenges and Limitations

Despite promising preclinical data, several barriers delay the clinical translation of actinomycete-derived antifungal compounds. Key challenges include large-scale cultivation, limited metabolite yield, structural complexity and batch-to-batch variability. Additionally, toxicity concerns—particularly off-target cytotoxicity—necessitate rigorous safety assessment. Pharmacokinetic limitations, such as poor oral bioavailability, rapid metabolism and limited tissue penetration, increasingly complicate clinical development. From a regulatory perspective, natural products face stringent requirements regarding standardization, reproducibility and quality control. Addressing these translational hurdles through formulation optimization, semi-synthetic modification and advanced delivery systems is essential for advancing actinomycete-derived antifungals toward clinical uses [25, 41].

3.11.1 Cultivation Difficulties of Actinomycetes

One of the primary challenges in researching actinomycetes for antifungal uses is their cultivation in laboratory settings. Actinomycetes, particularly those from the *Streptomyces* genus, often need specific growth conditions that mimic their natural environments. Several actinobacterial strains are slow increasing and include complex nutritional requirements, which complicates their cultivation. Additionally, strains may not grow adequately on standard media, leading to underrepresentation of potentially useful varieties [42]. The difficulty in culturing these microorganisms can result in the loss of valuable strains that may produce unique metabolites with antifungal characteristics.

3.11.2 Limited Understanding of Metabolite Biosynthesis

A significant challenge involves limited understanding of the biosynthesis pathways of antifungal metabolites produced by actinomycetes. Advances in genomics and bioinformatics have resulted in investigating genes and enzymes involved in secondary metabolite production. However, many biosynthetic gene clusters (BGCs) are still under-characterized or "silent" in laboratory conditions, complicating the identification and production of novel compounds [25]. Unraveling the complexities of these biosynthetic pathways is critical for enhancing metabolite production and downstream development of therapeutic agents.

3.11.3 Resistance Mechanisms in Fungal Pathogens

Another significant difficulty in development of actinomycete-derived antifungal therapies includes increasingly complex resistance mechanisms demonstrated by fungal pathogens. As the clinical use of antifungals expands, fungi evolve and acquire resistance often through multidrug resistance mechanisms, including efflux pumps and biofilm formation [31]. Understanding these resistance mechanisms is vital for developing effective and sustainable antifungal strategies. Actinomycete metabolites must be screened against a wide array of resistant strains to assess their potential as viable treatments, which needs comprehensive research approaches.

3.11.4 Regulatory Hurdles

The path from discovery to clinical use for natural products, including actinomycete-derived metabolites, is interrupted with regulatory challenges. The approval process for novel drugs can be prolonged and complicated, involving extensive preclinical and clinical trials to assess safety and efficacy [43]. This rigorous process can prevent research funding and investment in natural

product development, particularly for smaller biotech companies and academic institutions. Addressing these regulatory barriers needs strategic partnerships and funding opportunities to support the translational pathway of promising actinomycete-derived compounds.

3.11.5 Cost and Time Constraints

Developing novel antifungal agents from actinomycetes can be cost and time-intensive. The cost associated with traditional drug development, including screening, optimization and clinical trials, can be prohibitive. Additionally, the lengthy timelines needed for research often limit the ability to bring novel drugs to market quickly. The emergence of newly resistant fungal species highlights pressing needs of antifungal drugs; thus, enhancing the efficiency of research and development processes is critical [44].

3.11.6 Formulation Development Challenges for Actinomycete-Derived Antifungal Metabolites

A major translational barrier in developing actinomycete-derived metabolites, as antifungal therapeutics, is formulation optimization. Many natural products, particularly polyketides and macrolides, demonstrate poor aqueous solubility, chemical instability and susceptibility to rapid degradation, which significantly limit their clinical use. For example, although amphotericin B is highly effective, this drug needs lipid-based formulations to decrease nephrotoxicity and improve pharmacological performance [45].

Natural metabolites frequently present challenges, including low bioavailability, limited mucosal penetration (particularly relevant for OPC) and instability under physiological pH conditions. Advanced drug delivery systems such as liposomal carriers, nanoemulsions, polymeric nanoparticles and mucoadhesive gels have shown promise in enhancing solubility, targeted delivery and sustained release of antifungal compounds [45]. These approaches are particularly important for OPC, where local retention time and epithelial penetration are critical determinants of therapeutic success. However, formulation development increases manufacturing complexity and production costs, which may limit scalability and accessibility. Therefore, rational formulation design must be integrated early in the drug development pipeline to ensure therapeutic efficacy and industrial feasibility.

3.11.7 Pharmacokinetic and Pharmacodynamic Profiling

Comprehensive pharmacokinetic and pharmacodynamic profiling is essential before clinical translation of actinomycete-derived compounds. Many promising metabolites show potent in vitro activity but fail to maintain adequate in vivo exposure because of poor absorption, rapid metabolism, high plasma-protein binding, or insufficient mucosal penetration. For OPC, local drug distribution to the oral mucosa and saliva is particularly important. Therefore, future studies should integrate early PK/PD assessment with exposure-response modeling to optimize dosing and reduce the risk of resistance development [46].

3.11.8 Regulatory Pathways and Approval Challenges for Natural Products

Natural-product-derived therapeutics also face regulatory challenges related to compound characterization, reproducibility, toxicity assessment, manufacturing consistency, and quality control. For actinomycete-derived metabolites, batch-to-batch variability during microbial fermentation may complicate standardization. Standardized fermentation protocols, genomic validation of biosynthetic pathways, semi-synthetic optimization, and early regulatory consultation may help facilitate clinical development [47].

3.12 Further Directions

Future investigations should emphasize the genome-guided identification, functional assessment, and translational advancement of antifungal metabolites derived from actinomycetes. Techniques such as high-throughput sequencing, genome mining, metagenomics, and CRISPR-based methods can be utilized to uncover and activate dormant biosynthetic gene clusters that might produce new antifungal substances [25, 44]. These strategies must be combined with metabolomics, antifungal screening, and mechanistic tests targeting resistant and biofilm-forming Candida strains. Additionally, enhanced cultivation techniques, co-culture methods, and high-throughput screening systems could increase the availability of rare or previously uncultivated actinomycetes [42, 48]. Future research should also concentrate on rational combination therapies with existing antifungal drugs, standardized synergy testing, and fostering closer collaboration among microbiologists, clinicians, and pharmaceutical developers to hasten clinical translation [37, 42].

3.12.1 Clinical Pipeline and Translational Advancement of Actinomycete-Derived Antifungals

Although metabolites derived from actinomycetes exhibit significant potential in preclinical studies, only a limited number have advanced to later stages of clinical development. The success of antifungal agents like amphotericin B and echinocandin-related compounds, which originate from microbial natural products, illustrates the viability of this approach [49]. Nonetheless, newly discovered actinomycete metabolites still need enhancements in pharmacokinetics, toxicity evaluations, scalable production, formulation processes, and regulatory compliance before they can be used clinically. Consequently, future efforts should integrate the discovery of natural products with semi-synthetic improvements, enhanced delivery mechanisms, and early-stage translational strategies to transform promising antifungal metabolites into therapies suitable for clinical application [40, 44, 49].

4. Conclusion

The increasing clinical burden of antifungal resistance, particularly for OPC, highlights the urgent need of innovative and mechanistically distinct therapeutic strategies. While actinomycetes have long been recognized as prolific producers of bioactive secondary metabolites, their potential in antifungal drug development is comparatively uninvestigated. The evidence synthesized in this review indicates that actinomycete-derived compounds offer not only structural diversity but also opportunities to target biofilms, modulate resistance pathways and enhance the efficacy of available antifungal agents.

However, advancing these findings toward clinical translation needs a further structured and priority-driven research framework. Further investigations should move beyond investigatory screening and focus on (i) systematic assessment against resistant and biofilm-forming clinical isolates, (ii) integration of genome mining with functional validation to activate silent biosynthetic gene clusters and (iii) standardized synergy studies to define rational combination regimens. Importantly, preclinical development must incorporate pharmacodynamic modeling, toxicity profiling and formulation strategies tailored to mucosal delivery systems linked to OPC.

An equally critical problem is the implementation of resistance evolution studies to assess durability of antifungal activity and anticipate adaptive responses. Bridging natural product discovery with translational pharmacology, bioinformatics-guided compound prioritization and scalable production platforms is essential during drug development. Therefore, actinomycetes should be viewed not merely as a historical source of antimicrobials but as a strategic platform for

614 next-generation antifungal innovation. A coordinated, mechanism-oriented translationally aligned
615 research agenda includes the potential to transform actinomycete metabolites from promising
616 experimental agents into clinically viable therapeutics capable of addressing drug-resistant fungal
617 infections.

618 **Declaration of AI and AI-Assisted Technologies in the Writing Process**

619 The authors declare that Artificial Intelligence (AI) and AI-assisted technologies were exclusively
620 used during the primary search and literature gathering of this study. No AI tools were used for
621 data interpretation, text generation, drafting and editing of the manuscript. The final content was
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631 All data were used in the review.

632 **Author contributions**

633 I. H., Carried out the searches, gathered and analyzed data and prepared the preliminary version
634 of the manuscript; Z. J., proposed the study idea, supervised the study and edited the manuscript;
635 Y. N., provided technical assistance and edited the manuscript; A. S., proposed the study idea,
636 supervised the study and edited the manuscript; R. M. N. F., proposed the study idea, supervised
637 the study and edited the manuscript. All the authors read and approved the final version of the
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Conflict of interest

The authors declare no conflict of interest.

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