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EMERGING INSIGHTS INTO BIOFILM-FORMING CANDIDA SPECIES IN VULVOVAGINAL CANDIDIASIS: CLINICAL AND THERAPEUTIC PERSPECTIVES

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ABSTRACT

Vulvovaginal candidiasis (VVC) is a highly prevalent mucosal fungal infection harming females globally. A key factor in its recurrence is the ability of Candida species, particularly C. albicans, to form biofilm, and recently, there has been a significant rise in infection caused by non-albicans Candida (NAC) species is now recognized as a major contributor to recurrent vulvovaginal candidiasis (RVVC), treatment failure, and antifungal resistance. Key molecular mechanisms that cause antifungal resistance in biofilm-associated isolates include point mutations and overexpression of the target lanosterol 14-α-demethylase (ERG11), upregulation of efflux pumps (CDR1/CDR2 & MDR1), extracellular matrix (ECM) mediated drug sequestration, persistent cell formation, and phenotypic shifts. Advances in species identification (MALDI-TOF, ITS sequencing), rapid molecular diagnostics, and assays to detect biofilm production and resistance mechanisms are reforming clinical practice. Therapeutic strategies under active examination include optimized antifungal combinations, efflux pump modulators, biofilm dispersal agents, host-directed therapies (probiotics, immune modulators), antimicrobial peptides, and novel drug delivery systems (nanoparticles, intravaginal formulations). This review creates recent evidence on biofilm-associated Candida in VVC, molecular drivers of azole resistance focusing on ERG11 mutation/overexpression and efflux pumps diagnostic methods, evolving therapeutic options, and future research priorities.

KEYWORDS: Vulvovaginal candidiasis; Candida species; Biofilm; Azole resistance; ERG11; Efflux pump; antifungal therapy.

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INTRODUCTION

Vulvovaginal candidiasis (VVC) is the main common fungal infection in females, with millions affected worldwide [1]. It is a fungal infection predominantly seen in women of reproductive stage and results from the excessive growth of Candida species in the vaginal environment. The characteristic symptoms include thick, curd-like vaginal discharge, vulvar itching, pain during sexual intercourse (dyspareunia), burning sensation during urination (dysuria), swelling, and redness of the vulvovaginal area.

Epidemiological studies suggested that around 70–75% of sexually practicing women will incident at least one incident of VVC throughout their life span [2]. Among them, nearly 50% may suffer from recurrent infections, while around 5–10% develop recurrent vulvovaginal candidiasis (RVVC) [3,4]. Candida albicans is responsible for the majority of cases (85–90%) [5], However, non-albicans Candida (NAC) species, including Candida glabrata, Candida tropicalis, Candida krusei, and Candida parapsilosis, are also recognized as causative agents [6].

Several predisposing factors have been associated with VVC, including pregnancy (55%), prolonged use of broad-spectrum antibiotics (8%), uncontrolled diabetes, oral contraceptive use, immunosuppression, and HIV infection [7,9].

Approximately three-quarters of females of reproductive age are involved in at least one occurrence of vulvovaginal candidiasis (VVC), and nearly half of these females develop recurrent infections [10,11]. Vaginal colonization by Candida species is observed in about 20% of healthy women, with prevalence rising to nearly 30% during pregnancy [12]. The infection tends to be more frequent and difficult to manage in pregnant women due to hormonal and physiological changes in the urogenital tract that favor fungal proliferation [13,14].

Recent studies indicate that Candida infections during pregnancy may be associated with opposing motherly and newborn consequences, including preterm labor, untimely rupture of membranes, chorioamnionitis, and, in rare cases, congenital cutaneous candidiasis [15,16]. The frequency of vaginal Candida colonization among pregnant females varies between 10–50% [17], raising concern because vertical transmission can occur in 25–65% of cases, potentially resulting in invasive neonatal candidiasis [18,19].

The common antifungal drugs used for treating fungal diseases such as flucytosine, fluconazole, amphotericin B, voriconazole, clotrimazole, nystatin, Caspofungin, and ketoconazole. Although these drugs are widely used, they come with limitations, including toxicity, high treatment expenses, and the appearance of resistant Candida strains. Among these, azole antifungals remain the most effective due to their potent activity and oral bioavailability [20].

The extensive utilization of fluconazole in infection control protocol for Reproductive Tract and Sexually Transmitted Infections (RTI/STIs), especially in vaginal drug tools, has been linked to recurrence as well as therapeutic failures, especially infection caused by non-albicans Candida species. So, early preclusion, precise analysis, and appropriate therapy of VVC are vital to stop difficulties such as iliac seditious illness, infertility, ectopic pregnancy, pelvic abscesses, impulsive miscarriage, and catamenial irregularities [21]. Additionally, Candida biofilm formation significantly contributes to resistance against standard antifungal medications, complicating both in vitro susceptibility testing and clinical management [22,23].

The spectrum of antifungal activity varies across fungal species, much like the differences in antibiotic susceptibility observed among bacteria. Generally, Candida albicans, C. dubliniensis, and C. tropicalis are vulnerable to most antifungal agents commonly used in the management of fungemia. In comparison, C. glabrata often displays reduced vulnerability, whereas Candida krusei demonstrates intrinsic resistance to fluconazole. Additionally, C. parapsilosis typically shows lower sensitivity to echinocandins (Table 1) [24].

Table 1. Intrinsic susceptibility profiles of common fungal pathogens

Abbreviations: S – Susceptible; I – Intermediate; R – Resistant.

Fungal Species	Amphotericin	Echinocandins	Fluconazole	Itraconazole	Voriconazole	Posaconazole	5-Flucytosine	Terbinafine
Candida albicans	S	S	S	S	S	S	S	-
Candida glabrata	S	S	I-R*	S-I-R*	S-I-R*	S-I-R*	S	-
Candida krusei	S	S	R	I-R	S–I–R*	S-I-R*	R	-
Candida parapsilosis	S	S-I	S	S	S	S	S	-
Candida tropicalis	S	S	S	S	S	S	S	-
Saccharomyces cerevisiae	S	S	I-R*	S-I-R*	S–I–R*	S-I-R*	S	-
Cryptococcus spp.	S	R	S**	S	S	S	S	-
Trichosporon spp.	S-I-R	R	I-R	I-R	S	S	R	-
Fusarium spp.	S	R	R	R	S-I-R	S-I-R	R	S-I-R

*Wild-type strains of C. glabrata and C. krusei, even absence of acquired resistance procedures, exhibit lower azole susceptibility than C. albicans. Variability in MIC methods often causes these isolates to span S, I, and R categories. Both species are sub-optimally targeted by azoles, according to EUCAST, and fluconazole resistance frequently correlates with cross-resistance to other azoles. ** Hetero-resistance to fluconazole has been observed in Cryptococcus neoformans.

The rising antifungal resistance among Candida species is largely attributed to the improper and excessive use of antifungal medications, particularly in the treatment of vulvovaginal candidiasis (VVC). Although azole derivatives are the preferred treatment, frequent and repeated use of fluconazole has contributed to the emergence of multidrug-resistant strains and persistent diseases, raising a growing public health concern. Tackling antifungal resistance requires the quick discovery and development of new therapeutic compounds with strong antifungal activity, as current options are limited by factors such as availability issues, decreased effectiveness, toxicity, poor tolerability, and potential drug–drug interactions [24,25].

Epidemiology and species distribution in VVC

Although proportions vary by topography and patient population, recent surveillance and clinical studies indicate that C. albicans still predominates overall. Still, NAC species now account for a rising share of isolates in many regions, sometimes exceeding 20–40%, depending on the cohort (e.g., higher NAC prevalence in hospitalized or azole-exposed populations) [25,26]. C. glabrata and C. krusei are particularly important because of inherent or frequent azole reduced susceptibility [26,27].

Accurate species identification is life-threatening because species differ in antifungal susceptibilities and biofilm phenotypes. Modern tools, such as ITS sequencing and MALDI-TOF MS, significantly improve identification speed and accuracy compared to traditional biochemical methods [27,28].

Biofilm formation in Candida and relevance to VVC

Biofilm formation in Candida species is an extremely structured and dynamic process that develops through distinct stages, including adhesion, early proliferation, maturation with extensive extracellular matrix (ECM) accumulation, and eventual dispersal. In the context of vulvovaginal candidiasis, biofilms are characterized by a dense three-dimensional network and ECM components such as polysaccharides, proteins, and extracellular DNA, which act as a physical and chemical barrier to antifungal penetration. During this process, the upregulation of adhesins, including members of the ALS family and HWP1, along with transcriptional regulators such as BCR1 and EFG1, enhances mucosal adherence and persistence of infection. Biofilms are also enriched with persister cells, metabolically dormant populations that display tolerance to high concentrations of antifungal agents and can initiate recurrent infections following therapy. Furthermore, biofilm-associated transcriptional reprogramming upregulates drug-resistance genes, such as efflux pumps, and modifies sterol biosynthesis pathways, thereby reducing drug efficacy. Clinical isolates from VVC patients often demonstrate considerable variability in biofilm biomass and structural complexity, with stronger biofilm formers reliably associated with elevated antifungal tolerance and an increased risk of repeated disease [28,29].

Molecular Basis of Antifungal Resistance in Biofilm-Forming Strains

Role of ERG11: ERG11 codes lanosterol 14-α-demethylase, the main enzymatic purpose of azole antifungals. Alterations in this gene play a central role in azole resistance.

ERG11: Point Mutations: Specific point mutations in ERG11 can reduce the binding affinity of azoles, thereby diminishing the drug's effectiveness. Generally reported substitutions include Y132F and K143R, among others identified across multiple studies. These mutations are frequently observed in clinical isolates displaying azole resistance [29,30].

Overexpression of ERG11: Transcriptional overexpression of ERG11 leads to an increased abundance of the target enzyme, which in turn reduces the inhibitory effects of azoles. Such overexpression may result from gain-of-function mutations in upstream transcriptional controllers or from chromosomal changes, such as trisomy of chromosome 5 or the formation of an isochromosome carrying ERG11 [30,31].

Genomic Amplification and Combined Mechanisms: In several isolates, ERG11 mutations are observed in combination with efflux pump overexpression, creating high-level resistance that is more difficult to treat. Genomic amplification further enhances resistance by increasing the gene quantity of ERG11 [31,32].

Efflux Mechanisms: ABC Transporters (CDR1/CDR2) and (MFS) Transporter (MDR1)

The overexpression of efflux pumps is the single most well-established mechanism contributing to antifungal resistance in Candida species, particularly among isolates exposed to azoles or those associated with biofilm formation. These transport systems actively export antifungal compounds from the cell, reducing their intracellular concentration and consequently diminishing drug effectiveness.

ABC Transporters (CDR1/CDR2): ATP-binding cassette (ABC) transporters belonging to the CDR family are responsible for the broad-spectrum efflux of azole antifungals. Enhanced expression of CDR1 and CDR2 has been closely associated with multi-azole resistance in both biofilm-forming cells and resistant clinical isolates [32,33].

MFS Transporter (MDR1): The major facilitator superfamily (MFS) transporter MDR1 provides suggestively to fluconazole resistance. Its expression is frequently regulated by gain-of-function mutations in transcriptional activators such as Mrr1, resulting in continuous overexpression [33,34].

Recent investigations on vulvovaginal candidiasis have demonstrated increased appearance levels of CDR1 and MDR1 in fluconazole-resistant recurrent isolates, underscoring their vital role in treatment failure and clinical relapse [34,35].

Biofilm-specific mechanisms

Aside from canonical genetic mechanisms, biofilms confer drug tolerance through ECM-binding/sequestration of drug, changed metabolic states, oxygen and nutrient gradients that reduce drug activity, and formation of persistent cells. These mechanisms are phenotypic and reversible yet clinically important because they require different therapeutic approaches (higher drug exposure, combination therapy, or biofilm dispersal) [35,36].

Species identification and resistance mechanism profiling

MALDI-TOF MS: MALDI-TOF MS has appeared as a fast, high-throughput, also cost-efficient technique for finding medical yeast isolates, including Candida species implicated in vulvovaginal candidiasis. This method operates by generating protein mass spectral profiles that are matched against established reference databases, enabling species-level identification within minutes. Compared with traditional culture-based techniques, MALDI-TOF MS offers significantly shorter processing times,

enhanced accuracy, and improved capability to distinguish closely related species, for example, Candida albicans and various non-albicans Candida (NAC) species. In recent years, this technology has become increasingly integrated into clinical diagnostic laboratories for the detection and monitoring of VVC, serving as a dependable first-line tool for routine species identification [36,37].

Sequencing Approaches (ITS and D1/D2 Regions): Molecular sequencing continues to serve as the gold standard for accurate identification of Candida species, particularly when isolates exhibit unusual phenotypic traits or when detailed epidemiological analysis is required. The internal transcribed spacer (ITS) area of ribosomal DNA and the D1/D2 domain of the 28S rRNA gene are the most frequently sequenced targets for Candida identification. ITS sequencing offers excellent resolution for differentiating most clinically significant species, while D1/D2 sequencing complements it by clarifying complex taxonomic relationships. These molecular techniques are especially useful for identifying rare or emerging Candida species that may be misclassified by conventional phenotypic or biochemical methods. Furthermore, sequencing data provides valuable insights for molecular epidemiology, supporting strain differentiation, outbreak surveillance, and the monitoring of resistant Candida lineages [37,38].

Biofilm Detection and Phenotyping

In Vitro Assays: Various standardized in vitro techniques are commonly used to estimate the biofilm-forming competence of Candida species. The crystal violet assay is typically employed to quantify total biofilm biomass based on dye retention, whereas the XTT reduction assay measures the metabolic activity of biofilm cells. Other approaches include counting colony-forming units (CFUs) from detached biofilms to determine viable cell numbers. Advanced imaging methods, such as confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM), enable complete imaging of biofilm structure, thickness, and architecture. These assays are essential in research for both qualitative and quantitative evaluation of biofilm formation.

Proteomic and Mass Spectral Signatures: Emerging proteomic techniques have begun to identify unique biofilm-associated molecular signatures. MALDI-TOF MS, beyond its application in species identification, has shown spectral differences between planktonic and biofilm phenotypes. Such mass spectral profiling suggests the potential to rapidly infer biofilm-associated proteomes, providing insight into functional changes connected with biofilm growth. While currently at a research stage, these approaches may eventually facilitate rapid clinical detection of biofilm-forming isolates [38,39].

Detecting Resistance Mechanisms

Antifungal Susceptibility Testing (AFST): Conventional antifungal susceptibility testing, performed corresponding to CLSI (Clinical and Laboratory Standards Institute) and EUCAST (European Committee on Antimicrobial Susceptibility Testing) strategies, remains the primary method for evaluating resistance patterns in clinical Candida isolates. AFST determines the minimum inhibitory concentration (MIC) of antifungal agents, providing critical information for guiding treatment, particularly in recurrent or drug-resistant cases of vulvovaginal candidiasis.

Molecular Assays: Molecular methods have significantly advanced resistance mechanism profiling. PCR and Sanger sequencing enable the detection of ERG11 mutations that alter azole drug binding. Quantitative PCR (qPCR) allows for expression analysis of resistance-associated genes such as ERG11, CDR1/CDR2, and MDR1, offering insights into gene overexpression-mediated resistance. Furthermore, multiplex or targeted molecular panels can accelerate the detection of known resistance markers, improving turnaround times compared to culture-based approaches. Rapid PCR-based diagnostics for vaginitis have demonstrated higher sensitivity for Candida detection compared to conventional culture methods, and continuing advancements suggest these platforms could be expanded to include resistance markers, thus bridging species identification with resistance profiling in a single assay [39,40].

Clinical implications

Treatment failure and RVVC: Biofilm-forming isolates with combined ERG11 modifications and efflux pump up-regulation are more likely to persist after standard single-dose azole regimens, contributing to RVVC [34,35].

Species-guided therapy: identification of NAC species (e.g., C. glabrata, C. krusei) signals the clinician to anticipate reduced azole susceptibility and to consider other agents or longer regimens [26,27].

Therapeutic Perspectives and Emerging Strategies

Optimizing Current Antifungals: Present antifungal agents remain the cornerstone of VVC management, but their effectiveness can be limited in biofilm-associated or recurrent infections. Echinocandins exhibit potent activity against many Candida species; however, systemic administration results in limited vaginal penetration. Topical or locally delivered formulations are under investigation and may hold potential for treating biofilm-associated infections. Polyenes such as nystatin and amphotericin B retain activity against many azole-resistant isolates, making them valuable alternatives in cases of RVVC. Additionally, dose and duration modifications, including prolonged or repeated treatment courses and, in some cases, higher drug exposure, may be necessary to overcome biofilm-mediated tolerance and ensure clinical resolution.

Combination Therapy and Adjuvants: Combination therapy is increasingly being explored to improve treatment outcomes in resistant VVC. In vitro studies indicate that combinations such as azole plus echinocandin or azole plus polyene can act

synergistically against biofilm-forming Candida. While clinical evidence remains limited, systematic reviews suggest that combination regimens may outperform monotherapy in managing resistant isolates, particularly in recurrent cases [40,41].

Targeting Efflux Pumps and Regulatory Networks: Efflux pump inhibitors (EPIs) represent a promising strategy to restore azole susceptibility. By blocking ABC and MFS transporters, EPIs can reduce drug extrusion and increase intracellular antifungal concentrations in vitro. Translating this approach to safe and effective intravaginal therapy is an active area of research [40,41].

Biofilm Dispersal and ECM-Targeting Approaches: Targeting the extracellular matrix (ECM) of biofilms can enhance antifungal penetration and efficacy. Enzymatic agents such as DNases or glucanases, surfactants, and inhibitors of adhesins (e.g., ALS inhibitors) have demonstrated the ability to disrupt the biofilm structure in experimental models, thereby improving drug delivery and antifungal activity [35,36].

Novel Antimicrobials and Delivery Systems: Emerging strategies include the development of antimicrobial peptides (AMPs) and synthetic peptidomimetics with anti-biofilm activity, which are being formulated for vaginal delivery in preclinical studies [41,42]. Nanoparticle-based and mucoadhesive intravaginal formulations are also under investigation to enhance local drug concentration and penetration into biofilms. Immunomodulatory approaches, including vaccines, aim to boost mucosal immunity and reduce Candida colonization, although these remain in early experimental phases. Probiotic interventions targeting the restoration of a Lactobacillus-dominated vaginal microbiome may help limit Candida overgrowth and recurrence. While clinical evidence is still emerging, these approaches show promise as adjunctive therapies in the management of VVC.

Diagnostic–therapeutic algorithm

Based on current evidence and expert recommendations, a practical approach to suspected or recurrent vulvovaginal candidiasis with concern for biofilm formation or antifungal resistance involves several key steps. First, diagnosis should be confirmed through microscopy and, whenever feasible, molecular testing to identify the specific Candida species. Rapid PCR-based panels offer enhanced sensitivity, while culture and antifungal susceptibility testing (AFST) are particularly important in recurrent or treatment-refractory cases. Species identification using MALDI-TOF MS or sequencing provides additional guidance for therapy selection [41,43].

In cases of recurrent infection or treatment failure, assessment of resistance mechanisms such as mutations in ERG11 and overexpression of efflux pumps (CDR1, CDR2, MDR1) should be conducted when resources permit, or patients should be referred to specialized diagnostic laboratories. Identification of these resistance factors may necessitate the use of alternative or combination antifungal therapies, including non-azole agents or topical polyenes [31,32]. Additionally, adjunctive approaches, such as strategies to disrupt biofilms, probiotic supplementation, and improved local drug delivery, are being explored to enhance therapeutic outcomes and reduce the likelihood of recurrence.

Future research priorities

Future research in vulvovaginal candidiasis should focus on connecting laboratory findings with clinical outcomes. Standardized in vitro biofilm assays are needed, as current methodologies are heterogeneous, and correlating specific biofilm phenotypes with treatment failure remains a priority. Large, prospective studies are also acceptable to assess molecular resistance markers such as ERG11 mutations, efflux pump expression, and chromosomal alterations in well-characterized VVC cohorts, linking these findings to clinical outcomes. Additionally, the development and testing of safe and effective intravaginal formulations of novel therapeutics, including efflux pump inhibitors, antimicrobial peptides, and nanoparticle-based delivery systems, should be evaluated in randomized clinical trials. Finally, interventions targeting the vaginal microbiome to prevent recurrent VVC require rigorous controlled studies to determine efficacy, optimal strains, and treatment regimens.

CONCLUSION

Biofilm formation significantly impacts on both the pathogenic potential and fungicidal vulnerability of Candida species in vulvovaginal candidiasis (VVC). Key molecular mechanisms, including ERG11 mutations or overexpression and upregulation of efflux pumps, drive azole resistance and recurrent infections, and these mechanisms often coexist in clinical isolates. Enhancing diagnostic approaches through precise species identification, antifungal susceptibility testing (AFST) and molecular resistance panels combined with species-specific therapy and novel anti-biofilm interventions, represents the most effective strategy to reduce treatment failures and recurrent VVC (RVVC). Integrating standardized laboratory biofilm phenotyping with clinically validated antibiofilm strategies will be crucial to reduce recurrence and improve patient outcomes.

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