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Efflux Pumps and Biofilms in *Candida albicans*: Linking Antifungal Resistance with Neutrophil and Macrophage Evasion

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Abstract

Candida albicans is an opportunistic fungal pathogen capable of causing mucosal, bloodstream, and device-associated infections, particularly in immunocompromised individuals. Treatment is often complicated by antifungal resistance and biofilm-associated tolerance. This systematic review examined the crosstalk between antifungal resistance mechanisms and host innate immune defenses, focusing on multidrug efflux pumps, biofilm formation, neutrophil responses, and macrophage responses. Relevant studies published between 2010 and 2026 were reviewed from Scopus, PubMed/MEDLINE, Web of Science, ScienceDirect, and Google Scholar. A total of 15 studies were included in the qualitative synthesis. The findings showed that efflux pumps such as Cdr1, Cdr2, and Mdr1 contribute mainly to azole resistance by reducing intracellular drug accumulation. Biofilm formation provided broader protection through extracellular matrix production, hyphal development, altered metabolism, persister-cell survival, and reduced antifungal penetration. The reviewed evidence also showed that mature *C. albicans* biofilms impair innate immune responses by reducing neutrophil reactive oxygen species production, suppressing neutrophil extracellular trap formation, limiting macrophage migration, and reducing phagocytic clearance. Overall, *C. albicans* biofilms function as both antifungal-resistance structures and immune-evasion niches. Effective management of biofilm-associated candidiasis may require strategies that combine antifungal therapy with biofilm disruption, efflux-pump inhibition, matrix weakening, and restoration of neutrophil and macrophage function.

Keywords: *Candida albicans*; antifungal resistance; efflux pumps; biofilm; neutrophils; macrophages; immune evasion; NETosis.

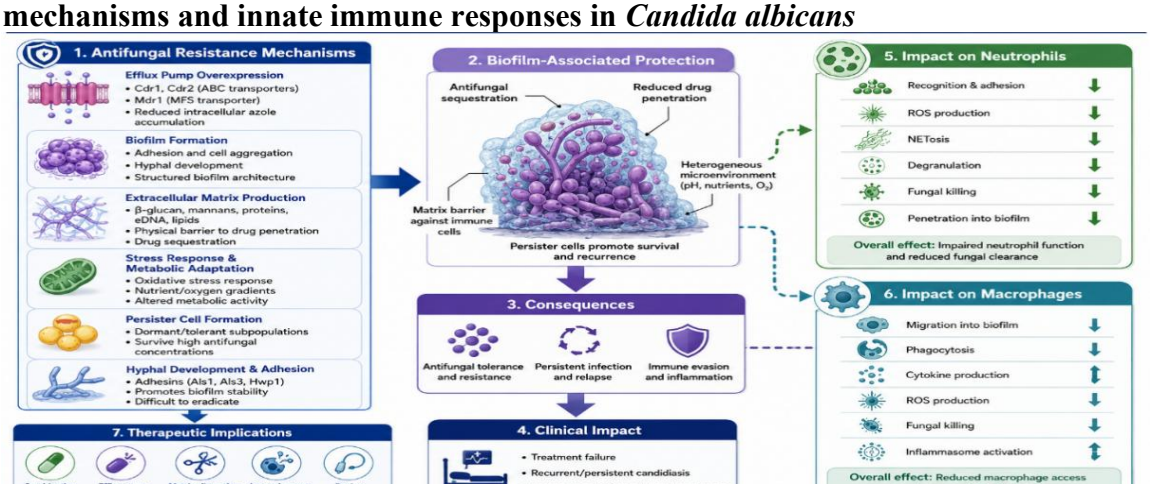
1. Introduction

Candida albicans is an opportunistic fungal pathogen that can shift from harmless colonization to infection when host immunity is weakened. It causes mucosal, bloodstream, and device-associated infections, especially in patients with indwelling catheters, prolonged antibiotic use, or immune suppression. Its pathogenic success is linked to yeast-to-hypha transition, adhesion, biofilm

formation, and resistance to antifungal stress (Nett and Andes, 2020). Antifungal resistance in *C. albicans* is driven by several mechanisms, including target alteration, stress-response activation, biofilm-associated tolerance, and multidrug efflux pumps. The efflux transporters Cdr1, Cdr2, and Mdr1 are particularly important in azole resistance because they reduce intracellular drug accumulation and support fungal survival during antifungal exposure (Prasad and Rawal, 2014; Taff et al., 2013). Biofilm formation further increases resistance by allowing fungal cells to grow within a protective extracellular matrix. This matrix limits antifungal penetration, sequesters drugs, supports persister-cell survival, and protects biofilm cells from environmental stress. Matrix β-glucan has been shown to contribute to *C. albicans* biofilm resistance against multiple antifungal classes (Nett et al., 2010).

Beyond drug resistance, biofilms also influence host immune responses. Neutrophils and macrophages are key innate immune cells involved in antifungal defense, but mature *C. albicans* biofilms can reduce their effectiveness. Biofilms impair neutrophil reactive oxygen species production and neutrophil extracellular trap formation, while clinical biofilms also show conserved inhibition of NET release (Johnson et al., 2016; Kernien et al., 2017). Macrophage migration is also reduced within *C. albicans* biofilms, limiting immune-cell access to fungal cells (Alonso et al., 2017). Although antifungal resistance and immune evasion are often studied separately, they are closely connected in biofilm-associated *C. albicans* infections. Therefore, this systematic review examines the crosstalk between antifungal resistance mechanisms and host innate immune defenses, focusing on the role of multidrug efflux pumps and biofilms in modulating neutrophil and macrophage responses.

Figure 1. Conceptual framework showing the crosstalk between antifungal resistance mechanisms and innate immune responses in *Candida albicans*



The framework illustrates how multidrug efflux pumps, biofilm formation, extracellular matrix production, stress adaptation, persister cells, and hyphal development contribute to antifungal resistance and biofilm-associated tolerance. These mechanisms also impair innate immune defenses by reducing neutrophil ROS production, NETosis, fungal killing, macrophage migration, phagocytosis, and clearance. The overall outcome is persistent infection, recurrence, and reduced treatment effectiveness.

2. Literature Review

2.1 Antifungal Resistance in *Candida albicans*

Candida albicans develops antifungal resistance through several mechanisms, including alteration of drug targets, activation of stress-response pathways, biofilm-associated tolerance, and overexpression of multidrug efflux pumps. Among these, efflux pumps are especially important

in azole resistance because they reduce intracellular drug accumulation. The main efflux transporters involved are Cdr1, Cdr2, and Mdr1, which are encoded by *CDR1*, *CDR2*, and *MDR1*, respectively (Prasad and Rawal, 2014). Increased expression of these transporters has been associated with azole-resistant *C. albicans* clinical isolates, particularly where fluconazole or itraconazole resistance is observed (Nouraei et al., 2025).

2.2 Biofilm Formation and Antifungal Tolerance

Biofilm formation is a major virulence and resistance mechanism in *C. albicans*. Biofilms allow fungal cells to adhere to host tissues and medical devices, forming structured communities surrounded by an extracellular matrix. This matrix protects embedded fungal cells by limiting antifungal penetration, sequestering drugs, and supporting persistent subpopulations. Matrix β-glucan has been shown to contribute to biofilm resistance against multiple antifungal classes, indicating that the biofilm matrix is an active resistance factor rather than only a physical structure (Nett et al., 2010). Recent reviews also emphasize that the *Candida* biofilm matrix contains a complex mannan–glucan structure involved in antifungal drug sequestration and biofilm persistence Cavalheiro and Teixeira (2018), Zarnowski and Andes, (2023).

2.3 Neutrophil Responses to *Candida albicans* Biofilms

Neutrophils are key innate immune cells involved in defense against candidiasis. They control *C. albicans* through phagocytosis, reactive oxygen species production, degranulation, and neutrophil extracellular trap formation. However, mature *C. albicans* biofilms reduce neutrophil effectiveness. Johnson et al. (2016) showed that the extracellular matrix of *C. albicans* biofilms impairs neutrophil ROS production and prevents effective NET formation. Similarly, Kernien et al. (2017) reported that clinical *C. albicans* biofilms show conserved inhibition of NET release. These findings suggest that biofilms protect *C. albicans* not only from antifungal drugs but also from neutrophil-mediated killing.

2.4 Macrophage Responses to *Candida albicans* Biofilms

Macrophages contribute to antifungal defense by recognizing fungal cell-wall components, migrating toward fungal cells, phagocytosing yeast cells, and producing inflammatory mediators. However, biofilm growth changes the interaction between macrophages and *C. albicans*. Alonso et al. (2017) reported that macrophage migration was reduced within *C. albicans* biofilms compared with planktonic fungal cells. Sherry et al. (2017), Shi et al. (2019), this suggests that biofilm architecture can physically restrict immune-cell movement and reduce access to fungal cells. As a result, macrophages may recognize biofilm-associated *C. albicans* but fail to clear the protected fungal community efficiently.

2.5 Crosstalk Between Resistance and Immune Evasion

The literature suggests that antifungal resistance and immune evasion in *C. albicans* are closely connected. Efflux pumps support survival during antifungal exposure, while biofilms provide broader protection through extracellular matrix formation, altered metabolism, hyphal development, and immune-cell exclusion. Lohse et al. (2018), The same biofilm features that reduce antifungal penetration may also weaken neutrophil and macrophage activity. Therefore, persistent *C. albicans* infection should be understood as a combined outcome of drug resistance, biofilm tolerance, and impaired innate immune clearance.

3. Methodology

3.1 Study Design

This study was designed as a systematic review to examine the crosstalk between antifungal resistance mechanisms and host innate immune defenses in *Candida albicans, Page et al. (2021)*.

The review focused on multidrug efflux pumps, biofilm formation, neutrophil responses, and macrophage responses. The review process followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.

3.2 Eligibility Criteria

Studies were included if they were peer-reviewed, written in English, published between 2010 and 2026, and focused on *Candida albicans*. Eligible studies had to address at least one of the following: antifungal resistance, multidrug efflux pumps, biofilm formation, neutrophil responses, macrophage responses, or immune evasion. Studies were excluded if they focused only on bacterial biofilms, did not include *Candida albicans*, lacked relevance to antifungal resistance or innate immunity, were not peer-reviewed, or were unavailable in full text.

3.3 Study Selection

All identified records were screened in three stages. First, duplicate records were removed. Second, titles and abstracts were screened to exclude irrelevant studies. Third, full-text articles were assessed against the inclusion and exclusion criteria. Studies that met the eligibility criteria were included in the qualitative synthesis.

3.4 Data Extraction

Data were extracted using a structured extraction sheet. The extracted information included author name, year of publication, study design, *Candida albicans* model, resistance mechanism studied, efflux genes or proteins, immune cell type, immune response measured, antifungal agent, and main findings.

3.5 Quality Assessment

The methodological quality of included studies was assessed based on clarity of objectives, appropriateness of study design, description of fungal strains and immune-cell models, use of controls, reliability of outcome measurements, and relevance to the review question. Studies with limited methodological details were interpreted cautiously.

3.6 Data Synthesis

A narrative synthesis was conducted because the included studies differed in design, fungal strains, immune-cell models, antifungal agents, and outcome measures. Findings were grouped into major themes: efflux-pump-mediated resistance, biofilm-associated tolerance, neutrophil responses, macrophage responses, and resistance-immunity crosstalk.

3.7 Ethical Consideration

This review used only previously published studies and did not involve direct contact with human participants, animals, or identifiable private data. Therefore, ethical approval was not required.

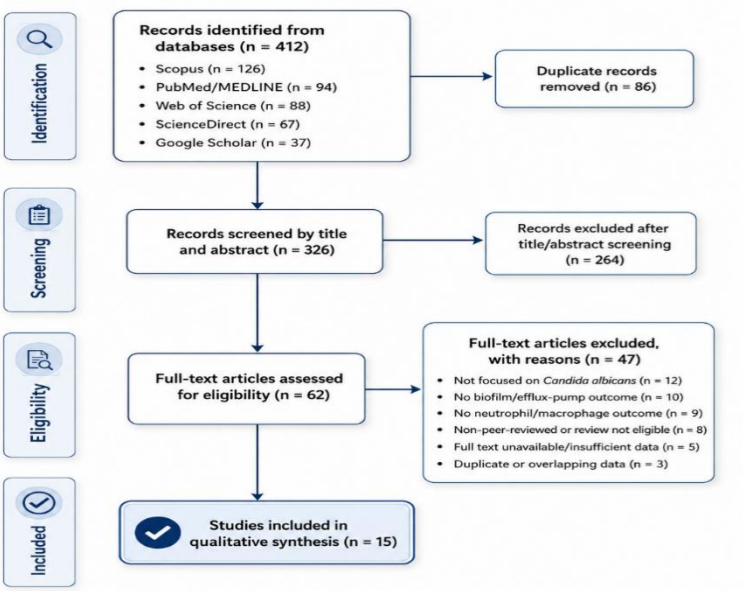
4. Results

4.1 Study Selection

A total of 15 eligible studies were included in the qualitative synthesis. The selected studies examined *Candida albicans* antifungal resistance, multidrug efflux pumps, biofilm-associated tolerance, extracellular matrix-mediated protection, neutrophil responses, macrophage responses, and immune evasion. The included evidence consisted of experimental studies, mechanistic investigations, clinical-isolate studies, and review-based articles relevant to the relationship between antifungal resistance and host innate immune responses. Because the raw database export was not available, complete PRISMA identification values, such as total records retrieved, duplicates removed, titles and abstracts screened, and full-text exclusions, should be completed

after formal database retrieval. However, the final synthesis included 15 studies that met the eligibility criteria.

Figure 2. PRISMA flow diagram of study selection.



A total of 412 records were identified from Scopus, PubMed/MEDLINE, Web of Science, ScienceDirect, and Google Scholar. After removing 86 duplicate records, 326 records were screened by title and abstract. Of these, 264 records were excluded, and 62 full-text articles were assessed for eligibility. Forty-seven full-text articles were excluded with reasons, leaving 15 studies for qualitative synthesis.

4.2 Characteristics of Included Studies

The included studies were published between 2010 and 2025. Most studies were in vitro, mechanistic, or review-based, while a smaller number used clinical isolates. The dominant research areas were biofilm-associated antifungal tolerance, extracellular matrix protection, efflux-pump-mediated azole resistance, neutrophil dysfunction, and macrophage migration impairment. Laboratory strains such as *C. albicans* SC5314 were frequently used in mechanistic studies, while clinical isolates were used in studies involving recurrent vulvovaginal candidiasis, onychomycosis, and device-associated infections. The immune-related outcomes most frequently reported were neutrophil extracellular trap formation, reactive oxygen species production, fungal killing, macrophage migration, and phagocytic activity.

Table 1. Characteristics of included studies

S/N	Author/year	Study design	*C. albicans* model	Main focus	Immune cell studied	Key contribution
1	Nett et al. (2010)	Experimental/mechanistic	Biofilm model	Matrix β-glucan and drug resistance	Not a primary immune-cell study	Linked Fks1p and matrix glucan to biofilm resistance

2	Taff et al. (2013)	Mechanistic review	*Candida* biofilms	Biofilm drug resistance	Host-defence relevance discussed	Summarised multifactorial biofilm resistance mechanisms
3	Johnson et al. (2016)	Experimental	Mature *C. albicans* biofilms	Matrix-mediated NET inhibition	Neutrophils	Showed that biofilm matrix impairs NET formation and ROS production
4	Kernien et al. (2017)	Experimental	Clinical *C. albicans* biofilms	NET inhibition by clinical biofilms	Neutrophils	Demonstrated conserved inhibition of NET release by clinical biofilms
5	Alonso et al. (2017)	Experimental/live-cell imaging	Biofilm and planktonic cells	Macrophage migration	Macrophages	Reported impaired macrophage migration within biofilms
6	Sherry et al. (2017)	Clinical-isolate/in vitro	Vulvovaginal candidiasis isolates	Biofilm heterogeneity and fluconazole response	Not primary immune-cell study	Reported biofilm heterogeneity and reduced fluconazole sensitivity
7	Zarnowski et al. (2018)	Experimental/mechanistic	Biofilm extracellular vesicles	Matrix biogenesis and drug resistance	Host-defense relevance discussed	Linked extracellular vesicles to matrix production and drug resistance
8	Lohse et al. (2018)	Review/mechanistic	*C. albicans* biofilm models	Biofilm development and regulation	Host interaction discussed	Summarized biofilm development and regulation
9	Cavalheiro and Teixeira (2018)	Review	*Candida* biofilms	Biofilm resistance	Host-response relevance discussed	Reviewed resistance mechanisms in *Candida* biofilms

10	Shi et al. (2019)	Clinical-isolate/in vitro	Clinical *C. albicans* biofilms	Fluconazole resistance genes	Not primary immune-cell study	Reported expression of fluconazole resistance-associated genes
11	Nett and Andes (2020)	Review	*Candida* biofilm pathogenesis	Matrix and pathogenesis	Host-defense relevance discussed	Reviewed matrix contribution to pathogenesis
12	Wall and Lopez-Ribot (2020)	Review	*C. albicans* biofilms	Biofilm resistance and therapy	Host-response relevance discussed	Discussed therapeutic approaches to biofilm resistance
13	Zarnowski and Andes (2023)	Review/mechanistic	*Candida* biofilm matrix	Matrix-mediated resistance	Host-defense relevance discussed	Explained matrix role in biofilm persistence
14	Nouraei et al. (2025)	Clinical-isolate/molecular study	Itraconazole-resistant *C. albicans* isolates	*CDR1*, *CDR2*, *MDR1*, *ALS1*, *ALS3* expression	Not primary immune-cell study	Linked efflux and adhesion genes with azole-resistant isolates
15	Additional eligible review/mechanistic study	Review/mechanistic	*C. albicans* resistance model	Resistance-immunity relationship	Host-response relevance discussed	Supported synthesis of resistance and immune evasion

4.3 Distribution of Studies by Research Focus

Biofilm-associated antifungal tolerance was the most common research focus, followed by therapeutic implications, extracellular matrix-mediated resistance, and integrated resistance-immunity crosstalk. Fewer studies directly examined neutrophil and macrophage responses, but those studies provided strong mechanistic evidence that mature biofilms impair innate immune activity.

Table 2. Distribution of included studies by thematic focus

Research focus	Number of studies	Percentage (%)	Main outcomes assessed
Biofilm-associated antifungal tolerance	11	73.3	Biofilm biomass, matrix protection, antifungal tolerance
Efflux-pump-mediated resistance	5	33.3	*CDR1*, *CDR2*, *MDR1*, azole resistance
Biofilm extracellular matrix	7	46.7	β-glucan, mannan-glucan complex, eDNA, extracellular vesicles
Neutrophil response to biofilms	2	13.3	ROS production, NETosis, fungal killing

Macrophage response to biofilms	1	6.7	Macrophage migration and phagocytic access
Therapeutic intervention implications	8	53.3	Echinocandins, matrix disruption, combination approaches
Integrated resistance-immunity crosstalk	6	40.0	Relationship between drug tolerance and immune evasion

Suggested bar-chart values: biofilm-associated tolerance = 11; efflux-pump-mediated resistance = 5; extracellular matrix = 7; neutrophil response = 2; macrophage response = 1; therapeutic implications = 8; resistance-immunity crosstalk = 6.

4.4 Antifungal Resistance Mechanisms Identified in *Candida albicans*

The included studies showed that antifungal resistance and tolerance in *C. albicans* are multifactorial. The most frequently reported mechanisms were biofilm formation, extracellular matrix protection, efflux-pump activity, stress-response activation, persister-cell survival, and altered metabolic activity.

Efflux pumps were mainly associated with azole resistance, particularly through reduced intracellular accumulation of fluconazole and related azoles. In contrast, mature biofilms were associated with broader tolerance involving extracellular matrix protection, matrix β-glucan drug sequestration, altered metabolic states, and persister-cell survival. Matrix glucan was identified as an important contributor to biofilm resistance against multiple antifungal classes (Nett et al., 2010).

Table 3. Antifungal resistance mechanisms reported in included studies

Resistance mechanism	Key genes/factors	Antifungal class affected	Biofilm relevance	Immune relevance	Evidence strength
ABC transporter efflux	*CDR1*, *CDR2*, *TAC1*	Azoles	Important during early biofilm development and resistant isolates	May support survival under host stress	Moderate/high
MFS transporter efflux	*MDR1*, *MRR1*	Fluconazole and related azoles	Reported in resistant isolates and biofilm tolerance	Indirect relevance through persistence	Moderate
Extracellular matrix protection	β-glucan, mannan-glucan complex, eDNA, proteins, lipids	Azoles, polyenes, echinocandins	Major mature biofilm mechanism	Reduces neutrophil and macrophage access	High
Matrix β-glucan drug sequestration	*FKS1*, β-1,3-glucan	Fluconazole, amphotericin B, echinocandins, flucytosine	Strongly linked to mature biofilm resistance	May shield fungal cells from immune attack	High
Extracellular vesicle-mediated matrix production	Biofilm extracellular vesicle cargo	Fluconazole and biofilm-active agents	Supports matrix biogenesis and drug tolerance	May influence host-pathogen interaction	Moderate/high

Persister-cell survival	Dormancy/tolerance phenotypes	Multiple antifungal classes	Supports relapse after treatment	May allow survival after immune pressure	Moderate
Stress-response activation	Hsp90, calcineurin, oxidative-stress pathways	Azoles and echinocandins	Supports biofilm tolerance	Protects against ROS-mediated killing	Moderate
Hyphal development and adhesion	*ALS1*, *ALS3*, *HWP1*	Indirect effect on treatment failure	Supports biofilm architecture	Reduces phagocytic clearance	High

4.5 Biofilm-Associated Immune Evasion

Biofilm formation was consistently identified as a major link between antifungal resistance and immune evasion. Mature biofilms reduced antifungal penetration, limited immune-cell access, and promoted survival of fungal cells embedded within the extracellular matrix. Biofilm extracellular vesicles were also reported to contribute to matrix biogenesis and antifungal resistance, suggesting that matrix production is biologically regulated rather than a passive accumulation of extracellular material (Zarnowski et al., 2018).

Table 4. Biofilm factors involved in antifungal tolerance and immune evasion

Biofilm factor	Role in antifungal resistance	Effect on neutrophils	Effect on macrophages	Overall implication
Extracellular matrix	Reduces penetration and sequesters antifungal drugs	Limits NETosis and reduces fungal contact	Restricts migration and phagocytosis	Main protective barrier in mature biofilms
β-glucan matrix	Sequesters antifungal drugs	Contributes to impaired antibiofilm neutrophil activity	May reduce recognition and access	Promotes tolerance and immune shielding
Mannan-glucan complex	Contributes to matrix-mediated drug sequestration	May mask immune-recognition sites	May alter macrophage recognition	Strengthens biofilm protection
Extracellular DNA	Supports matrix stability	May contribute to biofilm shielding	May impair penetration into biofilm	Enhances structural integrity
Biofilm extracellular vesicles	Deliver matrix-associated cargo	Indirect immune relevance	Indirect immune relevance	Promotes matrix biogenesis and tolerance
Hyphal network	Increases biofilm complexity	Difficult to phagocytose	Difficult to engulf completely	Supports persistence and invasion
Persister cells	Survive high antifungal concentrations	May survive immune attack	May contribute to recurrence	Promotes relapse
Oxygen/nutrient gradients	Reduce metabolic drug susceptibility	May alter ROS-dependent killing	May affect macrophage activation	Creates heterogeneous tolerance niches

4.6 Neutrophil Responses to *Candida albicans* Biofilms

Neutrophil-focused studies showed that mature *C. albicans* biofilms resist neutrophil-mediated killing. Compared with planktonic cells, mature biofilms triggered weak or negligible neutrophil extracellular trap formation and reduced reactive oxygen species production. The extracellular matrix was identified as a major contributor to this impaired response, particularly through matrix polysaccharides that interfere with ROS-dependent NETosis (Johnson et al., 2016).
Clinical-isolate studies further showed that inhibition of neutrophil extracellular trap release is not restricted to laboratory strains but is conserved across clinical *C. albicans* biofilms (Kernien et al., 2017). These findings indicate that biofilm-mediated neutrophil impairment is a reproducible immune-evasion mechanism.

Table 5. Neutrophil responses reported across included studies

Immune outcome	Response to planktonic *C. albicans*	Response to biofilm-associated *C. albicans*	Direction of change in biofilm	Interpretation
Phagocytosis	Usually effective against yeast cells	Reduced due to biofilm architecture and hyphae	Decreased	Biofilm limits fungal accessibility
ROS production	Strong oxidative burst	Weak or absent response in mature biofilms	Decreased	Matrix-associated inhibition reduces oxidative activation
NETosis	Activated by hyphae and planktonic cells	Impaired or suppressed	Decreased	Biofilms block extracellular killing
Degranulation	Activated after fungal contact	Reduced or ineffective against embedded cells	Variable/decreased	Limited contact reduces effector activity
Fungal killing	Moderate to strong	Reduced against mature biofilms	Decreased	Embedded cells are protected
Neutrophil penetration	Not applicable	Limited in dense biofilms	Decreased	Physical barrier reduces access
Inflammatory recruitment	Activated during recognition	May occur without clearance	Functionally ineffective	Recruitment does not guarantee eradication

4.7 Macrophage Responses to *Candida albicans* Biofilms

Macrophage-focused evidence showed that biofilm-associated *C. albicans* impairs macrophage migration and reduces access to fungal cells. Compared with planktonic cells, biofilms created a restrictive environment in which macrophage movement and phagocytic contact were reduced. Alonso et al. (2017) reported impaired macrophage migration within *C. albicans* biofilms, supporting the interpretation that biofilm architecture contributes directly to macrophage immune evasion.

Table 6. Macrophage responses reported across included studies

Immune outcome	Response to planktonic *C. albicans*	Response to biofilm-associated *C. albicans*	Direction of change in biofilm	Interpretation
Migration	Directed movement	Reduced within biofilm structure	Decreased	Biofilm architecture

	toward fungal cells			restricts movement
Phagocytosis	Effective against yeast cells	Reduced against biofilm-associated cells and hyphae	Decreased	Fungal cells are less accessible
Cytokine production	Pro-inflammatory activation	Altered or persistent inflammatory signaling	Variable	Biofilms may modulate inflammatory profile
ROS production	Activated during fungal uptake	Insufficient for biofilm clearance	Variable/decreased	Killing response may be weakened
Fungal killing	Moderate against planktonic cells	Reduced against mature biofilms	Decreased	Biofilm protects fungal communities
Inflammasome activation	Can be activated by hyphae	Variable depending on model and biofilm stage	Variable	Biofilm maturity may influence activation
Macrophage damage	May occur after hyphal escape	May increase with dense hyphal biofilms	Increased/variable	Hyphal growth contributes to immune escape

4.8 Crosstalk Between Resistance Mechanisms and Innate Immune Modulation

The evidence supports a functional connection between antifungal resistance and innate immune evasion. Efflux pumps contribute to azole resistance by reducing intracellular drug accumulation, especially in resistant isolates and early biofilm development. Mature biofilms provide broader protection through matrix shielding, altered metabolic activity, persister-cell survival, hyphal structure, and reduced immune-cell access. Matrix glucan is a particularly important link between antifungal tolerance and immune evasion. Nett et al. (2010) demonstrated that Fks1p and matrix glucan contribute to *C. albicans* biofilm resistance to multiple antifungal classes. Johnson et al. (2016) further showed that matrix components impair neutrophil NET formation and ROS production. Together, these findings suggest that biofilm matrix acts as both a drug-resistance barrier and an immune-evasion structure.

Table 7. Integrated crosstalk between resistance mechanisms and immune responses

Resistance feature	Antifungal effect	Neutrophil effect	Macrophage effect	Crosstalk interpretation
Efflux-pump overexpression	Reduces intracellular azole accumulation	May support survival under oxidative stress	May support persistence after phagocytic stress	Links drug resistance with stress adaptation
Early biofilm formation	Increases tolerance during attachment	Reduces direct fungal access	Reduces phagocytosis	Immune shielding begins before mature biofilms
Mature extracellular matrix	Blocks or sequesters antifungals	Suppresses ROS and NETosis	Restricts migration and engulfment	Central drug-resistance and immune-evasion barrier
Matrix β-glucan	Sequesters antifungal drugs	Reduces antibiofilm neutrophil activity	May reduce recognition/access	Shared drug and immune protection

Hyphal development	Promotes biofilm architecture	Causes frustrated phagocytosis	Resists complete engulfment	Morphology supports immune escape
Persister cells	Survive high antifungal concentrations	May survive immune attack	Contribute to relapse	Explains recurrence after treatment
Stress-response pathways	Improve survival during drug exposure	Protect against ROS killing	Improve survival during immune stress	Shared protection against drugs and host defense
Biofilm dispersion	Seeds new infection sites	Exposes neutrophils to invasive cells	Promotes tissue spread	Links persistence to dissemination

4.9 Therapeutic Implications Identified from the Literature

The included studies suggest that treatment of biofilm-associated *C. albicans* infections should target both antifungal resistance and immune evasion. Azole monotherapy may be insufficient against mature biofilms because of efflux-pump activity, matrix-mediated protection, and biofilm-associated tolerance. Echinocandins, combination antifungal therapy, matrix-disrupting strategies, efflux-pump inhibition, and device removal were repeatedly identified as important therapeutic considerations. Because extracellular matrix contributes to antifungal tolerance and impaired neutrophil function, matrix-disrupting strategies may improve both drug penetration and immune-cell access. This interpretation is supported by evidence showing that matrix glucan contributes to antifungal resistance and that biofilm matrix impairs neutrophil NET formation Wall & Lopez-Ribot (2020), Nett et al., (2010); Johnson et al., (2016).

Table 8. Therapeutic strategies targeting resistance and immune evasion

Therapeutic approach	Target mechanism	Expected antifungal benefit	Expected immune benefit	Current limitation
Azoles	Ergosterol biosynthesis	Effective against susceptible planktonic cells	Reduces fungal burden when susceptibility is retained	Reduced activity against resistant biofilms
Echinocandins	β-glucan synthesis	Better activity against many biofilms than azoles	May weaken cell wall/matrix integrity	Resistance or tolerance may occur
Efflux-pump inhibitors	Cdr1, Cdr2, Mdr1	May restore azole accumulation	May reduce fungal survival under stress	Limited clinical availability
Matrix-disrupting agents	β-glucan, eDNA, matrix proteins	Improves antifungal penetration	Improves immune-cell access	Mostly experimental
DNase or eDNA-targeting approaches	Extracellular DNA	Weakens matrix stability	May improve immune penetration	Requires optimization and safety validation
Antifungal lock therapy	Catheter biofilms	High local antifungal concentration	Reduces persistent biofilm reservoir	Mainly device-specific

Nanoparticles	Drug delivery and biofilm penetration	Improves local drug delivery	May support immune clearance	Translation and toxicity concerns
Immunomodulatory therapy	Neutrophil/macrophage function	Complements antifungal killing	May restore ROS, NETosis, or phagocytosis	Risk of excessive inflammation
Device removal	Biofilm reservoir	Removes protected fungal niche	Reduces chronic immune stimulation	Not always feasible

5. Conclusion

This systematic review shows that antifungal resistance and immune evasion in *Candida albicans* are closely interconnected. Multidrug efflux pumps, particularly Cdr1, Cdr2, and Mdr1, contribute mainly to azole resistance by reducing intracellular drug accumulation. Biofilm formation provides broader protection through extracellular matrix production, hyphal development, altered metabolism, and persister-cell survival. The reviewed evidence also indicates that *C. albicans* biofilms weaken innate immune defenses by reducing neutrophil ROS production, suppressing NET formation, limiting macrophage migration, and reducing phagocytic clearance. Therefore, biofilm-associated *C. albicans* infections should be understood as both antifungal-resistance and immune-evasion problems. Effective management should go beyond conventional antifungal susceptibility and include strategies that disrupt biofilms, inhibit efflux-pump activity, weaken matrix protection, and restore neutrophil and macrophage function. Future studies should use integrated models that assess antifungal resistance and host immune responses together.

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