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Worldwide emergence of fluconazole-resistant *Candida parapsilosis*: current framework and future research roadmap

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See **Online** for appendix

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Abstract

Candida parapsilosis is one of the most commen causes of life-threatening candidaemia, particularly in premature neonates, individuals with cancer of the haematopoietic system, and recipients of organ transplants. Historically, drug-susceptible strains have been linked to clonal outbreaks. However, worldwide studies started since 2018 have reported severe outbreaks among adults caused by fluconazole-resistant strains. Outbreaks caused by fluconazole-resistant strains are associated with high mortality rates and can persist despite strict infection control strategies. The emergence of resistance threatens the efficacy of azoles, which is the most widely used class of antifungals and the only available oral treatment option for candidaemia. The fact that most patients infected with fluconazole-resistant strains are azole-naive underscores the high potential adaptability of fluconazole-resistant strains to diverse hosts, environmental niches, and reservoirs. Another concern is the multidrug-resistant and echinocandin-tolerant *C parapsilosis* isolates, which emerged in 2020. Raising awareness, establishing effective clinical interventions, and understanding the biology and pathogenesis of fluconazole-resistant *C parapsilosis* are urgently needed to improve treatment strategies and outcomes.

Introduction

Candida parapsilosis is the second to fourth most common species causative of candidaemia, depending on patient age and geographic location.[1] Although initially considered fully susceptible to fluconazole, the emergence of fluconazole-resistant *C parapsilosis* has been reported in a growing number of countries.[2] Fluconazole-resistant strains raise concern for multiple reasons, including the high mortality of patients with invasive disease.[3–5] In many low-income and middle-income countries, fluconazole is the most widely used antifungal for treating *Candida* infections, and alternative treatments are costly or not readily available.[6,7] The presence of fluconazole-resistant isolates jeopardises azole-based treatment strategies that do not rely on the parenteral administration of echinocandin drugs as first-line agents. Fluconazole-resistant strains might replace susceptible *C parapsilosis* isolates and cause severe outbreaks that could persist despite the application of strict infection control strategies.[3–5] Since 2020, studies have reported echinocandin-resistant and multidrug-resistant *C parapsilosis* strains,[8–10] which pose a severe public health threat given the restricted number of antifungal drugs currently available for clinical use. This comprehensive Review discusses the current knowledge on the emergence of fluconazole-resistant *C parapsilosis* and offers solutions and new frameworks to minimise the spread of clonal outbreaks caused by fluconazole-resistant *C parapsilosis* isolates.

C parapsilosis epidemiology and infection risk factors

The *C parapsilosis* species complex encompasses three species, including *C parapsilosis* sensu stricto (*C parapsilosis* hereafter), and two closely related species that are less clinically prevalent: *Candida orthopsilosis* and *Candida metapsilosis.*[1] *Lodderomyces elongisporus* is another species within this complex.[11] *C parapsilosis* is among the most prevalent species that causes candidaemia worldwide, representing more than 20% of *Candida* species found in blood cultures in Brazil, Argentina, Peru, Spain, Russia, and China, and more than 30% in Türkiye, Greece, Croatia, Romania, South Africa, Nigeria, and Paraguay. *C parapsilosis* is also the third or fourth most common causative species of candidaemia in many other areas of the world (figure 1A, appendix pp 13–38).

C parapsilosis infections primarily occur in patients admitted to intensive care units, and in specific populations, such as neonates,[12] individuals with transplants,[13] people with COVID-19,[7] and people with cancer.[4] In these groups, *C parapsilosis* infections have been reported as the leading cause of candidaemia. Outbreaks associated with this species have been increasingly documented since 2018. The outbreaks are caused either by contaminated intravenous solutions[14] or by horizontal transmission from the environment to health-care workers' hands and then to patients.[15] *C parapsilosis* fungaemia is associated with a mortality rate of approximately 25%, which is lower than the mortality associated with *Candida albicans* fungaemia.[16] However, even higher mortality rates have been reported in patients with malignancy and cardiovascular prosthetic devices.[17]

Known risk factors for *Candida* infections include previous abdominal surgery, total parenteral nutrition, fungal colonisation, central venous catheters, use of broad-spectrum antibiotics, septic shock, and renal replacement therapy.[18,19] Patients with an independent risk for *C parapsilosis* candidaemia (*vs C albicans* candidaemia) in multivariate modelling include neonates, recipients of transplants, recipients of parenteral nutrition, and individuals with a history of antifungal therapy use.[13] Among children, mechanical ventilation has been described as an independent risk factor for *C parapsilosis* candidaemia,[20] and central venous catheters were a risk factor for *C parapsilosis* candidaemia among patients with solid or haematological cancers.[21] Although scarce data exist on specific risk factors for fluconazole-resistant *C parapsilosis* candidaemia, diabetes has been reported as a major clinical risk factor.[22] In a study from Brazil, diabetes was the only independent risk condition for acquiring candidaemia due to fluconazole-resistant *C parapsilosis*.[23] Pinhati and colleagues hypothesised that the physical contact between health-care workers and people with diabetes to appropriately monitor and treat this disease, as well as the fact that diabetic patients are prone to *Candida* colonisation, might explain this finding.[23] Large-scale international studies are required to validate these findings and identify further risk factors that might predispose individuals to develop candidaemia due to fluconazole-resistant strains.

Burden of fluconazole-resistant *C parapsilosis* and resistance mechanisms

Historically, *C parapsilosis* was known as a fluconazole-susceptible *Candida* species and drug resistant strains were rarely recorded before 2010. In 2004, a large outbreak of fluconazole-resistant *C parapsilosis* was reported in a community hospital in MS, USA.[24]

Before 2019, scattered studies described a more than 10% prevalence of fluconazole-resistant among all *C parapsilosis* strains in many areas worldwide (figure 1B, appendix pp 13–38). In the past 3 years, fluconazole-resistant *C parapsilosis* strains expanded and outcompeted fluconazole-susceptible isolates in health-care settings[3,5] persisting in various hospital niches and continually causing sporadic outbreaks among azole-naive patients despite the application of strict infection control strategies.[4] A steady increase in the number of clonal fluconazole-resistant *C parapsilosis* strains has been recorded in many countries worldwide (figure 1C). Consistent with spread from 2018 onwards, a global collection of *Candida* isolates revealed the emergence of fluconazole-resistant *C parapsilosis* strains that carry the *ERG11*^{Y132F} mutation, with most isolates originating in Europe, especially in Italy.[25] Azole-naive patients who were severely ill from COVID-19 were particularly susceptible to acquiring fungaemia due to fluconazole-resistant *C parapsilosis* strains, with outbreaks reported in Brazil,[26,27] Spain,[28,29] Greece,[30] and Türkiye.[31] Therefore, the global ongoing COVID-19 pandemic that has exhausted intensive care unit capacities and strained infection control procedures might be fuelling the surge of infections caused by the spread of fluconazole-resistant *C parapsilosis* worldwide. Independent studies in cohorts from Türkiye,[3,5] Brazil,[4] France,[32] and the USA,[33] have found that, compared with fungaemia in patients infected with fluconazole-susceptible strains, individuals infected with fluconazole-resistant strains had higher mortality rates (50–63·8% *vs* 16·1–20%).[4,5] The mechanisms that underpin fluconazole resistance and echinocandin resistance in *C parapsilosis* are multifaceted, with drug target modifications being their major cause. Additional details regarding antifungal resistance mechanisms are discussed (figure 2, appendix p 3).

Fitness cost and resilience of fluconazole-resistant *C parapsilosis* isolates in a clinical setting

Given that drug-resistant pathogens typically harbour mutations in genes involved in essential cellular processes, it is plausible that drug-resistant isolates incur a fitness cost and therefore would be readily outcompeted by susceptible counterparts in the absence of antifungal drugs. Thus, the restriction of azole use might lead to a substantial reduction in the proportion of fluconazole-resistant *C parapsilosis* strains in outbreak scenarios. However, it is possible that fluconazole-resistant isolates might have similar, if not higher, fitness than their susceptible counterparts, for two reasons. First, in an outbreak, fluconazole-resistant *C parapsilosis* isolates are more likely than fluconazole-susceptible isolates to be transferred from one host to another. If fluconazole-resistant isolates have longer survival periods in human hosts than fluconazole-susceptible isolates, this property might provide these strains with a greater opportunity to adapt to host conditions through genomic changes. Examples of remarkable genomic plasticity accompanied by swift adaptation to host conditions have been identified in other *Candida* species.[34] This in-host adaptation is further highlighted by the fact that individuals infected with fluconazole-resistant isolates carrying *ERG11*^{Y132F} have significantly higher mortality rates than individuals infected with other strains.[3–5] Second, the majority of fluconazole-resistant *C parapsilosis* isolates are recovered from azole-naive patients.[3–5] These observations suggest that fluconazole-resistant strains might thrive in the absence of azole exposure and, therefore, that the restriction of azole use might not be sufficient to minimise the spread of fluconazole-resistant *C parapsilosis* strains

in health-care settings. Ex-vivo and in-vivo studies with outbreak isolates in experimental models will be required to quantify the fitness costs linked to emergence of phenotypic resistance.

Clinical implications of azole and echinocandin tolerance

In medical mycology, the terms tolerance and persistence are not precisely defined and often used interchangeably. Azole tolerance refers to the slow growth of a subpopulation of fungal cells (which makes for >1% of the total pupulation) in high concentrations (ie, >2 times minimum inhibitory concentration) of a fungistatic azole drug.[35,36] Azole tolerance has been extensively studied in *C albicans*, and studies have found that exposure to fluconazole can lead to an increase in the copy number of *ERG11* and *TAC1* via aneuploidy.[36] Studies show that *C parapsilosis* isolates with intermediate fluconazole MICs might develop phenotypic fluconazole resistance by increasing the *CDR1* copy number in the absence of any *TAC1* or *MRR1* mutations.[37] Therefore, it is tempting to speculate that such mechanisms increase the azole-tolerant subpopulation, which ultimately increases the likelihood of the emergence of fluconazole resistance.

Echinocandin tolerance is technically and mechanistically different from azole tolerance, with echinocandin tolerance referring to the survival of a very small subpopulation (<1% depending on the timepoint and concentration used) of a given *Candida* species in high concentrations of a fungicidal drug (appendix p 4).

Heteroresistance refers to *C parapsilosis* isolates that contain a small population of resistant cells, typically 0·1% to 0·01%, among a large population of susceptible cells (typically 99·9% or higher). The heteroresistant phenotype can be detected by population analysis profiling assay, in which 10^5–10^6 cells are plated on medium containing a series of different drug concentrations, and survivor colonies at each specific concentration are enumerated. Individuals undergoing allogeneic haematopoietic cell transplantation colonised with susceptible strain (in the gut) alone were much less likely to develop candidaemia while on micafungin prophylaxis compared with patients with an allogenic haematopoietic cell transplantation colonised with heteroresistant isolates, who were more likely to develop breakthrough candidaemia.[38] The emergence and expansion of heteroresistant strains in the intestinal reservoir during micafungin prophylaxis and the isolation of paired heteroresistant isolates from the intestine and blood possibly explains why individuals with allogenic haematopoietic cell transplant colonised with *C parapsilosis* can develop breakthrough candidaemia while on micafungin prophylaxis (figure 3).[38]

Tools to dissect virulence and drug resistance in

C parapsilosis Genome analysis of *C parapsilosis* was first enabled by the reconstruction of a reference genome assembly over a decade ago and by continuous gene annotation made publicly available at the Candida Genome Database.[39,40] *C parapsilosis* has a diploid genome with very low heterozygosity, which consists of approximately 13 Mbp (haploid size) organised into eight chromosome pairs and a mitochondrial linear chromosome. The first population genomics study sequenced the genomes of several clinical and

environmental strains and compared them with the reference genome.[41] The comparison revealed very low sequence divergence between distant strains but a high variation in the copy number of key genes, such as agglutinin-like sequence proteins. Clinical isolates were non-monophyletic and thus did not cluster in phylogenetic analyses. Instead, clinical isolates were interspersed with environmental isolates in phylogenetic reconstructions. This pattern indicated recurrent transitions from the environment to the human host, and vice versa.[41] The study also provided indirect evidence of recombination among different strains, based on regions of unusually high genetic variability compared with related strains, and the presence of shared structural variants between unrelated strains. These findings questioned the long-held view that *C parapsilosis* was an asexual species, although direct experimental evidence of a sexual or parasexual cycle is still missing.

Beyond the insights provided by genome comparisons, whole-genome sequencing (WGS) facilitated insights into the molecular epidemiology of clinical strains of *C parapsilosis*, and allowed the identification of virulence and drug resistance attributes.[42] DNA typing of *C parapsilosis* isolates to define genotypic variation of strains responsible for outbreaks in hospitals has been generally done using locus-specific DNA typing approaches such as amplified fragment length polymorphisms or microsatellite polymorphisms.[3] These approaches have been useful in defining expansions of drug resistant strains after 2019.[3,5] Studies done after 2019, have exploited WGS to resolve clonality in hospital outbreaks[31,43] and coinfections by diverse lineages in patients with chronic candidiasis.[44] One of these studies directly compared isolated clusters derived by microsatellite DNA typing with genomic data and showed an overall consistency between results obtained from WGS and DNA typing.[45] As WGS prices decrease and analytical pipelines become standardised, the use of this technique will expand to allow in-depth epidemiological analyses, the dating of outbreaks and transmissions, the discovery of novel resistance-conferring mutations, and phenotype–genotype mapping through genome-wide association studies. However, the speed and simplicity of locus-specific typing approaches still offer an advantage for particular applications, such as routine clinical diagnostics, outbreak monitoring, and hospital surveillance.

Genetic transformation is required to infer the effect of genomic changes, especially single-nucleotide polymorphisms, identified by WGS or Sanger sequencing. Historically, site-directed mutagenesis has been fundamental to investigating the function of virulence factors and resistance determinants in *C parapsilosis*, which often cannot be directly extrapolated from *C albicans.*[1] Given *C parapsilosis* diploid genome, gene disruption used to require two rounds of transformation to independently replace the two alleles of a target gene with a recyclable selectable marker. The implementation of CRISPR-Cas9 for gene disruption in this species greatly streamlined this process,[46] because the Cas9 endonuclease introduces a double-strand break on both homologous chromosomes, which allows the simultaneous editing of both alleles in one step, increasing the efficiency of homologous recombination and reducing the operational time to generate mutants by at least 50% (appendix pp 4–5).

This advance accelerated the study of gene families associated with virulence in *C parapsilosis.*[40,47] One example is the *ALS* gene family, encoding cell wall proteins involved in adhesion and biofilm formation. CRISPR-Cas9 facilitated disrupting multiple *ALS* genes,

both in reference strains and highly adhesive strains to investigate their contribution to the adhesion process in vitro and in vivo.[48] In one case, CRISPR-Cas9 was used to simultaneously target all *ALS* paralogs in *C orthopsilosis*, a sibling species of *C parapsilosis*, proving that this technology is a crucial innovation for the study of entire gene families.[47]

CRISPR editing is also useful for easily introducing single-nucleotide polymorphisms in a target gene to experimentally validate whether specific amino acid changes result in drug resistance.[10,27,46] In 2022, Daneshnia and colleagues[27] used CRISPR-Cas9 to introduce *TAC1*^{L518F} into a type strain, ATCC 22019, and observed an eight-time increase in the minimum inhibitory concentration of fluconazole, due to the overexpression of the efflux pump *CDR1* (figure 2). Similarly, in 2023, CRISPR editing was used to experimentally validate that the *FKS1*^{S656P} allele confers pan-echinocandin resistance in *C parapsilosis*.[10] Additional information on CRISPR-Cas9 shortcomings and potential future directions is available (appendix pp 4–5).

Biofilm formation

C parapsilosis can form biofilms on biotic and abiotic surfaces. Biofilm is a community of cells adhered to a surface and encased in a protective extracellular matrix. Mature *C parapsilosis* biofilms confer protection from antifungal drugs and the host immune response.[1] Unlike *C albicans* biofilms, which contain yeast-form, pseudohyphal, and hyphal cells, *C parapsilosis* biofilms only contain yeast-form and pseudohypal cells.[1] Nonetheless, the ability to undergo morphological transitions is important for *C parapsilosis* biofilms. The biofilm-forming capacity of several *C parapsilosis* clinical strains have been shown to be directly related to their capacities for pseudohyphal cell growth.[49] The biofilm formation stages are displayed in the appendix (p 7).

Although the transcriptional circuitry that controls *C parapsilosis* biofilms has not yet been elucidated, the orthologs of the *C albicans* master biofilm regulators (*BCR1*, *BRG1*, *EFG1*, *FLO8, NDT80,* and *TEC1*) have been studied in *C parapsilosis*,[50–52] among which *BCR1, EFG1,* and *NDT80* were found to be required for biofilm formation.[52] Given the differences in the biofilm circuitry between *C albicans* and *C parapsilosis*, future research would benefit from elucidating the *C parapsilosis* biofilm circuitry using genome-wide binding and expression approaches.

There are many methods used to investigate *C parapsilosis* biofilms. The most valuable in-vitro biofilm research methods are arguably those that are somewhat representative of host niches. For example, high-glucose and lipid-rich media have been used in vitro to grow *C parapsilosis* biofilms, because these media are thought to contain clinically relevant ingredients that could elicit biofilm formation, similar to parenteral nutrition.[1] There is a positive correlation between *C parapsilosis* biofilm formation and glucose content. *C parapsilosis* has been shown to form more robust biofilms in media containing glucose, compared with media with no glucose content.[51] Animal models for investigating *C parapsilosis* biofilms in vivo have also been developed. The most established in vivo biofilm model is the rat central venous catheter model, which was first described for *C albicans*[53] and has been used to compare *C albicans* and *C parapsilosis* biofilms in vivo.[51]

C parapsilosis biofilms grown in Spider medium supplemented with 1% glucose appeared morphologically similar to in-vivo rat catheter biofilms.[51] Thus, this type of biofilm might be a good model for *C parapsilosis* biofilm studies in vitro.

C parapsilosis strains able to form biofilms were thought to be associated with outbreaks[1] and with a poorer clinical prognosis than isolates that do not form biofilms.[54] However, clinical studies published in 2020–22 suggested that fluconazole-resistant *C parapsilosis* strains causing severe outbreaks produce thin biofilms[3,27] and are associated with a poorer prognosis.[3,4] In a study focused on infant *C parapsilosis* bloodstream infections, genes associated with biofilm formation had lower expression in the gut of infants compared with other growth conditions (eg, pure culture).[49] A study published in 2021 showed that *C albicans* strains locked in the yeast form were better at colonising mucosal surfaces, disseminating, and adhering to epithelial and endothelial cells than the wild-type strain, which is possibly the result of improved metabolic adaptation and proliferation.[55] All these studies suggest that although biofilm formation is an important virulence factor for *C parapsilosis*, possibly needed for survival and resilience on surfaces, there could be an evolutionary trade-off between biofilm formation and adaptation in some host niches.

Immunology and microbiome

When *C parapsilosis* cells encounter phagocytes that patrol host mucosal surfaces, fungal cell wall polysaccharides activate C-type lectin receptor signalling in host cells. The best understood interaction is the activation of the mammalian C-type lectin receptor Dectin-1 (encoded by the *CLEC7A* gene) by fungal β-glucans. The *FKS1* gene, which is the target of echinocandin drugs, represents an essential component of the *C parapsilosis* β-glucans biosynthetic pathway. Following activation, CLRs transmit signals via the canonical Syk-CARD9 pathway to activate NF-κB and promote the production of proinflammatory cytokines, exemplified by tumour necrosis factor and neutrophil-recruiting chemokines.[56] This signalling pathway promotes the recruitment and activation of fungicidal effector cells at the portal of infection, primarily neutrophils and monocytes. In a murine systemic model of infection, deletion of either SYK or CARD9 signalling in haematopoietic cells led to enhanced *C parapsilosis* growth in the kidneys and brain, two important target organs of the disease. It is unknown whether azole-resistant or echinocandin-resistant strains have altered immune-activating properties, by virtue of drug exposure-dependent changes in the fungal cell wall composition.[57]

Although *C parapsilosis* is less virulent than *C albicans*, *C parapsilosis* encodes virulence attributes that facilitate invasive disease and nutrient acquisition in mammalian hosts. These virulence attributes include a lipase and a multicopper oxidase.[1,58,59] In addition, strain and morphologic variability contribute to infectious outcomes; these phenotypic differences might, in part, reflect differences in the expression of adhesin-encoding genes.[44]

C parapsilosis represents a component of endogenous fungal communities (ie, the mycobiota) in the human gastrointestinal tract. The relationship between *C parapsilosis* in the microbiota and the development of invasive disease is described (appendix pp 5–6).

Infection control strategies

Outbreaks of fluconazole-resistant *C parapsilosis* infections have been reported worldwide in the past decade, and the phylogenetic clustering of resistant strains that show similar genotypes strongly suggests horizontal transmission of infection.[5,22,32,60] *C parapsilosis* and *C auris* isolates can colonise the hands of health-care workers and medical devices and persist for long periods of time in the hospital environment.[1,61]

Antifungal stewardship should be implemented in all tertiary care hospitals not only to optimise costs and management of fungal infections, but also to reduce the unnecessary use of prophylactic and empirical antifungals.[62] Additionally, the incorporation of fungal biomarker testing into algorithms for selecting patients at a high risk for empirical therapy in intensive care units might reduce the unnecessary exposure of critically ill patients to fluconazole.[18]

Because nosocomial cross-contamination or transmission appears to be the main mechanism of fluconazole-resistant *C parapsilosis* dissemination, the adoption of strict infection prevention and control measures is required to stop and prevent the spread of this pathogen in health-care settings.[1,26] A clean environment is essential for patient safety, especially in periods of resistant pathogen outbreaks.[63] To control outbreaks of fluconazole-resistant *C parapsilosis* strains, it is urgent to reinforce training and the implementation of adherence to hand hygiene, transmission-based precautions, and meticulous cleaning and disinfection of the environment complemented by appropriate control of the cleaning effectiveness.[26,63] However, it is debated whether quaternary ammonium disinfectants are effective against fluconazole-resistant *C parapsilosis* isolates.[26,64] For example, chlorine-based disinfectants and hydrogen peroxide are recommended to combat *C auris* and *Clostridium* species.[65,66] Although contact precaution and cohort isolation have been effective for containment of *C auris* outbreaks,[67] patient isolation for fluconazole-resistant *C parapsilosis* infections is a controversial issue in infection control practice. This strategy can pose a serious economic and logistic burden, especially in health-care settings with scarce resources.

Treatment options for *C parapsilosis* with the emergence of fluconazole resistance

European guidelines have deviated from the general recommendation of using echinocandins as a first-line agent for invasive *Candida* infections, and have endorsed fluconazole as a first-line treatment for *C parapsilosis*.[68] However, US guidelines published in 2016 have recommended echinocandins as first-line treatment for infections caused by *C parapsilosis*.[69] With the emergence of fluconazole-resistant *C parapsilosis* in Europe, echinocandins or, as an alternative, liposomal amphotericin B are recommended for azole-resistant isolates. Other azoles, such as posaconazole, isavuconazonium sulfate, or voriconazole are therefore currently the only oral options for treatment of fluconazole-resistant *C parapsilosis*. However, use of azole-class drugs to treat infections caused by fluconazole-resistant isolates is an untested strategy, with most experts advocating use of a different class of drug.[70] In addition, oral or intravenous formulations of isavuconazonium sulfate or posaconazole are not readily available in many parts of the world.[71] The

2020 emergence of echinocandin-tolerant[27] and multidrug-resistant *C parapsilosis* strains[7,9] underscores the urgent need for new compounds with novel mechanisms of action for treating azole-resistant or multidrug-resistant *C parapsilosis* infections.[27] Currently, a number of new antifungals are in late stages of development and clinical trials. Ibrexafungerp, a novel echinocandin-like antifungal drug class, and oteseconazole, an azole with activity against azole-resistant *Candida* species, have in the last 2 years received US Food and Drug Administration approval as oral treatments for vulvovaginal candidiasis.[72] Ibrexafungerp shows in-vitro activity against fluconazole-resistant *C parapsilosis* isolates.[73] The US Food and Drug Administration has also granted orphan drug designation approval for miltefosine to treat invasive candidiasis.[73] Worryingly, miltefosine resistance has also been noted among clinical *C parapsilosis* isolates underpinned by an increased copy number of *RTA3*.[75] Rezafungin, a once a week echinocandin with an extended half-life, has shown promise in phase 3 studies and is expected to be launched for clinical use in Europe later in 2023.[72] Other drugs with novel mechanisms of action, such as fosmanogepix, might provide additional treatment options for resistant *C parapsilosis* in the future.[72] Collectively, these observations indicate the urgent need to establish effective antifungal treatment regimens against fluconazole-resistant, echinocandin-resistant, and multidrug-resistant *C parapsilosis* strains.

Conclusions

Fluconazole-resistant *C parapsilosis* has emerged rapidly, with increasing numbers of clonal outbreaks reported worldwide since 2018. To prevent the exacerbation of this issue to a global crisis, it is imperative to pursue an interdisciplinary approach that combines detailed molecular and virulence studies on *C parapsilosis* as well as conduct clinical trials of new antifungal drugs and implement effective infection control strategies. Despite the severity of the fluconazole-resistant outbreak in multiple countries, molecular assays with high sensitivity and specificity are unavailable for environmental screening and for skin swab sample applications. Nonetheless, such tools are subject to various challenges, namely the regional variability of the fluconazole-resistant clones carrying *ERG11* mutations, changing prevalence of specific clones, and emergence of new ones over time. Additionally, azole resistance involves multiple genes and some studies have noted that more than 60% of the fluconazole-resistant starins do not carry any *ERG11* mutations.[27] Application of in-vitro, ex-vivo, and in-vivo studies are warranted to understand the fitness cost of fluconazole-resistant isolated relative to fluconazole-susceptible counterparts in the presence or absence of fluconazole, which might guide azole use restriction in outbreak-stricken centres. Given that outbreak-derived fluconazole-resistant *C parapsilosis* isolates probably gain higher adaptability in the host, it is imperative to use omics studies to better understand the interaction of fluconazole-resistant and fluconazole-susceptible strains with the immune system. Application of WGS analyses offers notable potential by providing data on the mechanisms that underlie drug resistance, virulence, genotypic profiles, and identification of the infection source. To tackle heteroresistance and azole or echinocandin tolerance in clinical practice, developing rapid, convenient, time-saving, and cost-effective protocols differentiating tolerance levels is required. However, revealing such differences at the subpopulation level either requires extending the incubation time of standard antifungal

susceptibility testing (for azole tolerance) or using labour-intensive protocols relying on extensive colony forming unit counting.[35,36] Therefore, devising alternative methods might increase the enthusiasm of clinical centres to use such assays to not only shed light on the clinical importance of tolerance and heteroresistance, but also to examine whether there is consistency between in vitro and real-life generated data. Given that fluconazole-resistant *C parapsilosis* resides in hospital niches, the application of regular screening is imperative to further identify the outbreak source. Traditional infection control strategies might be ineffective in outbreak control and innovative strategies have yet to be investigated; therefore, in vitro testing of potent disinfectants using high-throughput platforms, followed by their application in outbreak-stricken centres, could offer promising alternatives. The effectiveness of cohorting people infected by fluconazole-resistant *C parapsilosis* to contain the nosocomial spread of this emergent pathogen should be investigated. Although from 2020 the emergence of clonal multidrug-resistant isolates has posed a serious threat to the treatment efficacy of both echinocandins and azoles, several new drugs and drug classes are currently in late stage clinical management that will provide effective options for treatment of fluconazole-resistant *C parapsilosis* infections. However, at this point, more in-vitro, in-vivo, and clinical trials comparing various novel antifungal drugs in areas experiencing outbreaks due to drug-resistant *C parapsilosis* isolates are needed. Finally, conducting local, national, and international epidemiological studies on a regular basis is essential to monitor the pattern of resistance emergenc**e**, and the application of WGS will be useful to elucidate the circulation of genotypes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Tóth R, Nosek J, Mora-Montes HM, et al. *Candida parapsilosis*: from genes to the bedside. Clin Microbiol Rev 2019; 32: e00111–18. [PubMed: 30814115]
2. Arastehfar A, Lass-Flörl C, Garcia-Rubio R, et al. The quiet and underappreciated rise of drug-resistant invasive fungal pathogens. J Fungi (Basel) 2020; 6: 138. [PubMed: 32824785]
3. Arastehfar A, Daneshnia F, Hilmioğlu-Polat S, et al. First report of candidemia clonal outbreak caused by emerging fluconazole-resistant *Candida parapsilosis* isolates harboring Y132F and/or Y132F + K143R in Turkey. Antimicrob Agents Chemother 2020; 64: e01001–20. [PubMed: 32690638]
4. Thomaz DY, de Almeida JNJ Jr, Sejas ONE, et al. Environmental clonal spread of azole-resistant *Candida parapsilosis* with Erg11-Y132F mutation causing a large candidemia outbreak in a Brazilian Cancer Referral Center. J Fungi (Basel) 2021; 7: 259. [PubMed: 33808442]

5. Arastehfar A, Hilmioğlu-Polat S, Daneshnia F, et al. Clonal candidemia outbreak by *Candida parapsilosis* carrying Y132F in Turkey: evolution of a persisting challenge. Front Cell Infect Microbiol 2021; 11: 676177. [PubMed: 33968809]
6. Pathadka S, Yan VKC, Neoh CF, et al. Global consumption trend of antifungal agents in humans from 2008 to 2018: data from 65 middle- and high-income countries. Drugs 2022; 82: 1193–205. [PubMed: 35960433]
7. Hoenigl M, Seidel D, Sprute R, et al. COVID-19-associated fungal infections. Nat Microbiol 2022; 7: 1127–40. [PubMed: 35918423]
8. Liang J, Macesic N, Blakeway L. Comment on: genetically related micafungin-resistant *Candida parapsilosis* blood isolates harbouring novel mutation R658G in hotspot 1 of Fks1p: a new challenge? J Antimicrob Chemother 2022; 77: 1790. [PubMed: 35325174]
9. Arastehfar A, Daneshnia F, Hilmioglu-Polat S, et al. Genetically related micafungin-resistant *Candida parapsilosis* blood isolates harbouring novel mutation R658G in hotspot 1 of Fks1p: a new challenge? J Antimicrob Chemother 2021; 76: 418–22. [PubMed: 33175162]
10. Ning Y, Xiao M, Perlin DS, et al. Decreased echinocandin susceptibility in *Candida parapsilosis* causing candidemia and emergence of a pan-echinocandin resistant case in China. Emerg Microbes Infect 2023; 12: 2153086. [PubMed: 36440795]
11. Lockhart SR, Messer SA, Pfaller MA, Diekema DJ. Geographic distribution and antifungal susceptibility of the newly described species *Candida orthopsilosis* and *Candida metapsilosis* in comparison to the closely related species *Candida parapsilosis*. J Clin Microbiol 2008; 46: 2659–64. [PubMed: 18562582]
13. Almirante B, Rodríguez D, Cuenca-Estrella M, et al. Epidemiology, risk factors, and prognosis of *Candida parapsilosis* bloodstream infections: case-control population-based surveillance study of patients in Barcelona, Spain, from 2002 to 2003. J Clin Microbiol 2006; 44: 1681–85. [PubMed: 16672393]
14. McCray E, Rampell N, Solomon SL, Bond WW, Martone WJ, O'Day D. Outbreak of *Candida parapsilosis* endophthalmitis after cataract extraction and intraocular lens implantation. J Clin Microbiol 1986; 24: 625–28. [PubMed: 3490490]
15. Qi L, Fan W, Xia X, et al. Nosocomial outbreak of *Candida parapsilosis* sensu stricto fungaemia in a neonatal intensive care unit in China. J Hosp Infect 2018; 100: e246–52. [PubMed: 29928941]
16. Barchiesi F, Orsetti E, Osimani P, Catassi C, Santelli F, Manso E. Factors related to outcome of bloodstream infections due to *Candida parapsilosis* complex. BMC Infect Dis 2016; 16: 387. [PubMed: 27507170]
17. Hirai Y, Asahata S, Ainoda Y, Goto A, Fujita T, Totsuka K. Nosocomial *Candida parapsilosis* candidemia: risk factors, antifungal susceptibility and outcome. J Hosp Infect 2014; 87: 54–58. [PubMed: 24698737]
18. Colombo AL, de Almeida Júnior JN, Slavin MA, Chen SCA, Sorrell TC. *Candida* and invasive mould diseases in non-neutropenic critically ill patients and patients with haematological cancer. Lancet Infect Dis 2017; 17: e344–56. [PubMed: 28774702]
19. Hoenigl M, Salmanton-García J, Egger M, et al. Guideline adherence predicts survival of candidemia in Europe: results from the ECMM *Candida* III multinational European study. Lancet Infect Dis 2023; published online Feb 15. 10.1016/S1473-3099(22)00872-6d (accessed April 1, 2023).
20. Dotis J, Prasad PA, Zaoutis T, Roilides E. Epidemiology, risk factors and outcome of *Candida parapsilosis* bloodstream infection in children. Pediatr Infect Dis J 2012; 31: 557–60. [PubMed: 22333703]
21. Hachem R, Hanna H, Kontoyiannis D, Jiang Y, Raad I. The changing epidemiology of invasive candidiasis: *Candida glabrata* and *Candida krusei* as the leading causes of candidemia in hematologic malignancy. Cancer 2008; 112: 2493–99. [PubMed: 18412153]
22. Thomaz DY, de Almeida JNJ Jr, Lima GME, et al. An azole-resistant *Candida parapsilosis* outbreak: clonal persistence in the intensive care unit of a Brazilian Teaching Hospital. Front Microbiol 2018; 9: 2997. [PubMed: 30568646]

23. Pinhati HMS, Casulari LA, Souza ACR, Siqueira RA, Damasceno CMG, Colombo AL. Outbreak of candidemia caused by fluconazole resistant *Candida parapsilosis* strains in an intensive care unit. BMC Infect Dis 2016; 16: 433. [PubMed: 27544427]
24. Clark TA, Slavinski SA, Morgan J, et al. Epidemiologic and molecular characterization of an outbreak of *Candida parapsilosis* bloodstream infections in a community hospital. J Clin Microbiol 2004; 42: 4468–72. [PubMed: 15472295]
25. Castanheira M, Deshpande LM, Messer SA, Rhomberg PR, Pfaller MA. Analysis of global antifungal surveillance results reveals predominance of *Erg11* Y132F alteration among azole-resistant *Candida parapsilosis* and *Candida tropicalis* and country-specific isolate dissemination. Int J Antimicrob Agents 2020; 55: 105799. [PubMed: 31520783]
26. Thomaz DY, Del Negro GMB, Ribeiro LB, et al. A Brazilian inter-hospital candidemia outbreak caused by fluconazole-resistant *Candida parapsilosis* in the COVID-19 era. J Fungi (Basel) 2022; 8: 100. [PubMed: 35205855]
27. Daneshnia F, de Almeida Júnior JN, Arastehfar A, et al. Determinants of fluconazole resistance and echinocandin tolerance in *C. parapsilosis* isolates causing a large clonal candidemia outbreak among COVID-19 patients in a Brazilian ICU. Emerg Microbes Infect 2022; 11: 2264–74. [PubMed: 36066554]
28. Trevijano-Contador N, Torres-Cano A, Carballo-González C, et al. Global emergence of resistance to fluconazole and voriconazole in *Candida parapsilosis* in tertiary hospitals in Spain during the COVID-19 pandemic. Open Forum Infect Dis 2022; 9: ofac605. [PubMed: 36467290]
29. Alcoceba E, Gómez A, Lara-Esbrí P, et al. Fluconazole-resistant *Candida parapsilosis* clonally related genotypes: first report proving the presence of endemic isolates harbouring the Y132F *ERG11* gene substitution in Spain. Clin Microbiol Infect 2022; 28: 1113–19. [PubMed: 35439634]
30. Mamali V, Siopi M, Charpantidis S, Samonis G, Tsakris A, Vrioni G. Increasing incidence and shifting epidemiology of candidemia in Greece: results from the first nationwide 10-year survey. J Fungi (Basel) 2022; 8: 116. [PubMed: 35205870]
31. Arastehfar A, Ünal N, Hoşbul T, et al. Candidemia among coronavirus disease 2019 patients in Turkey admitted to intensive care units: a retrospective multicenter study. Open Forum Infect Dis 2022; 9: ofac078. [PubMed: 35345665]
32. Fekkar A, Blaize M, Bouglé A, et al. Hospital outbreak of fluconazole-resistant *Candida parapsilosis*: arguments for clonal transmission and long-term persistence. Antimicrob Agents Chemother 2023; 95: e02036–20. [PubMed: 33593841]
33. Raghuram A, Restrepo A, Safadjou S, et al. Invasive fungal infections following liver transplantation: incidence, risk factors, survival, and impact of fluconazole-resistant *Candida* parapsilosis (2003–2007). Liver Transpl 2012; 18: 1100–09. [PubMed: 22577087]
34. Demers EG, Biermann AR, Masonjones S, et al. Evolution of drug resistance in an antifungal-naive chronic *Candida lusitaniae* infection. Proc Natl Acad Sci USA 2018; 115: 12040–45. [PubMed: 30389707]
35. Healey KR, Perlin DS. Fungal resistance to echinocandins and the MDR phenomenon in *Candida glabrata*. J Fungi (Basel) 2018; 4: 105. [PubMed: 30200517]
36. Gow NAR, Johnson C, Berman J, et al. The importance of antimicrobial resistance in medical mycology. Nat Commun 2022; 13: 5352. [PubMed: 36097014]
37. Branco J, Ryan AP, Pinto E Silva A, Butler G, Miranda IM, Rodrigues AG. Clinical azole cross-resistance in *Candida parapsilosis* is related to a novel MRR1 gain-of-function mutation. Clin Microbiol Infect 2022; 28: 1655.e5–8.
38. Zhai B, Liao C, Jaggavarapu S, et al. Echinocandin heteroresistance causes prophylaxis failure and facilitates breakthrough *Candida parapsilosis* infection. medRxiv 2022; published online May 29. 10.1101/2022.05.29.22275734 (preprint).
39. Skrzypek MS, Binkley J, Binkley G, Miyasato SR, Simison M, Sherlock G. The *Candida* Genome Database (CGD): incorporation of Assembly 22, systematic identifiers and visualization of high throughput sequencing data. Nucleic Acids Res 2017; 45: D592–96. [PubMed: 27738138]
40. Butler G, Rasmussen MD, Lin MF, et al. Evolution of pathogenicity and sexual reproduction in eight *Candida* genomes. Nature 2009; 459: 657–62. [PubMed: 19465905]

41. Pryszcz LP, Németh T, Gácser A, Gabaldón T. Unexpected genomic variability in clinical and environmental strains of the pathogenic yeast *Candida parapsilosis*. Genome Biol Evol 2013; 5: 2382–92. [PubMed: 24259314]
42. Schikora-Tamarit MÀ, Gabaldón T. Using genomics to understand the mechanisms of virulence and drug resistance in fungal pathogens. Biochem Soc Trans 2022; 50: 1259–68. [PubMed: 35713390]
43. Guinea J, Mezquita S, Gómez A, et al. Whole genome sequencing confirms *Candida albicans* and *Candida parapsilosis* microsatellite sporadic and persistent clones causing outbreaks of candidemia in neonates. Med Mycol 2021; 60: myab068. [PubMed: 34718724]
44. Gómez-Molero E, Willis JR, Dudakova A, et al. Phenotypic variability in a coinfection with three independent *Candida parapsilosis* lineages. Front Microbiol 2020; 11: 1994. [PubMed: 32983018]
45. Holland LM, Schröder MS, Turner SA, et al. Comparative phenotypic analysis of the major fungal pathogens *Candida parapsilosis* and *Candida albicans*. PLoS Pathog 2014; 10: e1004365. [PubMed: 25233198]
46. Lombardi L, Oliveira-Pacheco J, Butler G. Plasmid-based CRISPR-Cas9 gene editing in multiple *Candida* species. MSphere 2019; 4: e00125–19. [PubMed: 30867327]
47. Zoppo M, Luca MD, Villarreal SN, et al. A CRISPR/Cas9-based strategy to simultaneously inactivate the entire *ALS* gene family in *Candida orthopsilosis*. Future Microbiol 2019; 14: 1383–96. [PubMed: 31659913]
48. Zoppo M, Di Luca M, Franco M, et al. *CpALS4770* and *CpALS4780* contribution to the virulence of *Candida parapsilosis*. Microbiol Res 2020; 231: 126351. [PubMed: 31707298]
49. West PT, Peters SL, Olm MR, et al. Genetic and behavioral adaptation of *Candida parapsilosis* to the microbiome of hospitalized infants revealed by in situ genomics, transcriptomics, and proteomics. Microbiome 2021; 9: 142. [PubMed: 34154658]
50. Nobile CJ, Fox EP, Nett JE, et al. A recently evolved transcriptional network controls biofilm development in *Candida albicans*. Cell 2012; 148: 126–38. [PubMed: 22265407]
51. Mancera E, Nocedal I, Hammel S, et al. Evolution of the complex transcription network controlling biofilm formation in *Candida* species. eLife 2021; 10: e64682. [PubMed: 33825680]
52. Holland LM, Schröder MS, Turner SA, et al. Comparative phenotypic analysis of the major fungal pathogens *Candida parapsilosis* and *Candida albicans.* PLoS Pathog 2014; 10: e1004365. [PubMed: 25233198]
53. Andes D, Nett J, Oschel P, Albrecht R, Marchillo K, Pitula A. Development and characterization of an in vivo central venous catheter *Candida albicans* biofilm model. Infect Immun 2004; 72: 6023–31. [PubMed: 15385506]
54. Rajendran R, Sherry L, Nile CJ, et al. Biofilm formation is a risk factor for mortality in patients with *Candida albicans* bloodstream infection-Scotland, 2012–2013. Clin Microbiol Infect 2016; 22: 87–93. [PubMed: 26432192]
55. Dunker C, Polke M, Schulze-Richter B, et al. Rapid proliferation due to better metabolic adaptation results in full virulence of a filament-deficient *Candida albicans* strain. Nat Commun 2021; 12: 3899. [PubMed: 34162849]
56. Fan D, Coughlin LA, Neubauer MM, et al. Activation of HIF-1α and LL-37 by commensal bacteria inhibits *Candida albicans* colonization. Nat Med 2015; 21: 808–14. [PubMed: 26053625]
57. Hohl TM, Rivera A, Lipuma L, et al. Inflammatory monocytes facilitate adaptive CD4 T cell responses during respiratory fungal infection. Cell Host Microbe 2009; 6: 470–81. [PubMed: 19917501]
58. Pál SE, Tóth R, Nosanchuk JD, Vágvölgyi C, Németh T, Gácser A. A *Candida parapsilosis* overexpression collection reveals genes required for pathogenesis. J Fungi (Basel) 2021; 7: 97. [PubMed: 33572958]
59. Chakraborty T, Tóth Z, Tóth R, Vágvölgyi C, Gácser A. Iron metabolism, pseudohypha production, and biofilm formation through a multicopper oxidase in the human-pathogenic fungus *Candida parapsilosis.* MSphere 2020; 5: e00227–20. [PubMed: 32404511]
60. Souza ACR, Fuchs BB, Pinhati HMS, et al. *Candida parapsilosis* resistance to fluconazole: molecular mechanisms and in vivo impact in infected *Galleria mellonella* larvae. Antimicrob Agents Chemother 2015; 59: 6581–87. [PubMed: 26259795]

61. da Silva EM, Sciuniti Benites Mansano E, de Souza Bonfim-Mendonça P, et al. High colonization by *Candida parapsilosis* sensu stricto on hands and surfaces in an adult intensive care unit. J Mycol Med 2021; 31: 101110. [PubMed: 33450538]
62. Capoor MR, Subudhi CP, Collier A, Bal AM. Antifungal stewardship with an emphasis on candidaemia. J Glob Antimicrob Resist 2019; 19: 262–68. [PubMed: 31195147]
63. Han JH, Sullivan N, Leas BF, Pegues DA, Kaczmarek JL, Umscheid CA. Cleaning hospital room surfaces to prevent health care-associated infections: a technical brief. Ann Intern Med 2015; 163: 598–607. [PubMed: 26258903]
64. Fu L, Le T, Liu Z, et al. Different efficacies of common disinfection methods against *Candida auris* and other *Candida* species. J Infect Public Health 2020; 13: 730–36. [PubMed: 32005617]
65. Biswal M, Rudramurthy SM, Jain N, et al. Controlling a possible outbreak of Candida auris infection: lessons learnt from multiple interventions. J Hosp Infect 2017; 97: 363–70. [PubMed: 28939316]
66. Abdolrasouli A, Armstrong-James D, Ryan L, Schelenz S. In vitro efficacy of disinfectants utilised for skin decolonisation and environmental decontamination during a hospital outbreak with *Candida auris.* Mycoses 2017; 60: 758–63. [PubMed: 28872735]
67. Aldejohann AM, Wiese-Posselt M, Gastmeier P, Kurzai O. Expert recommendations for prevention and management of *Candida auris* transmission. Mycoses 2022; 65: 590–98. [PubMed: 35437832]
68. Cornely OA, Bassetti M, Calandra T, et al. ESCMID* guideline for the diagnosis and management of *Candida* diseases 2012: non-neutropenic adult patients. Clin Microbiol Infect 2012; 18 (suppl 7): 19–37. [PubMed: 23137135]
69. Pappas PG, Kauffman CA, Andes DR, et al. Clinical practice guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis 2016; 62: e1–50. [PubMed: 26679628]
70. Cornely OA, Hoenigl M, Lass-Flörl C, et al. Defining breakthrough invasive fungal infection-position paper of the mycoses study group education and research consortium and the European Confederation of Medical Mycology. Mycoses 2019; 62: 716–29. [PubMed: 31254420]
71. Salmanton-García J, Au WY, Hoenigl M, et al. The current state of laboratory mycology in Asia/Pacific: a survey from the European Confederation of Medical Mycology (ECMM) and International Society for Human and Animal Mycology (ISHAM). Int J Antimicrob Agents 2023; 61: 106718. [PubMed: 36640851]
72. Hoenigl M, Sprute R, Arastehfar A, et al. Invasive candidiasis: investigational drugs in the clinical development pipeline and mechanisms of action. Expert Opin Investig Drugs 2022; 31: 795–812.
73. Díaz-García J, Gómez A, Machado M, et al. Blood and intra-abdominal *Candida* spp. From a multicentre study conducted in Madrid using EUCAST: emergence of fluconazole resistance in *Candida parapsilosis*, low echinocandin resistance and absence of *Candida auris*. J Antimicrob Chemother 2022; 77: 3102–09. [PubMed: 36031723]
74. Bergin SA, Zhao F, Ryan AP, et al. Systematic analysis of copy number variations in the pathogenic yeast *Candida parapsilosis* identifies a gene amplification in RTA_3 that is associated with drug resistance. Mbio 2022; 13: e0177722. [PubMed: 36121151]

Search strategy and selection criteria

We searched PubMed, Google Scholar, and Web of Science using the keywords "*Candida parapsilosis*", "*Candida*", "resistance", "outbreak", "tolerance", "hetero-resistance", "biofilm", "epidemiology", "treatment", and "microbiome" to select relevant clinical and animal studies published in English between Jan 1, 2000 and Nov 10, 2022. We also searched citation lists of all relevant publications for additional references. Information on epidemiology, antifungal resistance, and outcome were extracted from the literature by FD, JNdAJ, and MI and verified in a second review of the articles by ME.

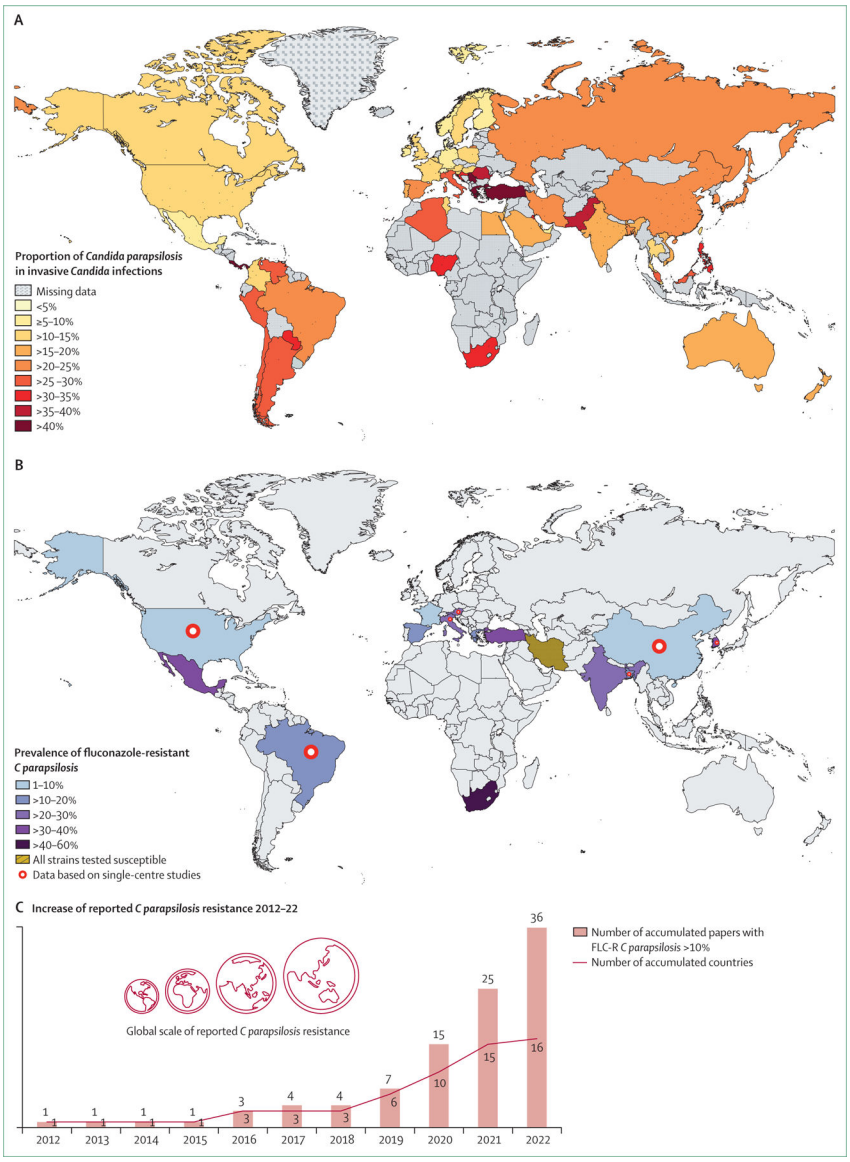

Figure 1: Burden and epidemiology of C parapsilosis worldwide.

(A) The worldwide epidemiology of *C parapsilosis*. (B) The burden of fluconazole resistant isolates. More details available in the appendix (pp 13–43). (C) Countries reporting *C parapsilosis* bloodstream isolates with fluconazole resistance more than 10% sharply increased during the COVID-19 pandemic, including some outbreaks with clonal dissemination of resistant isolates (appendix pp 39–43).

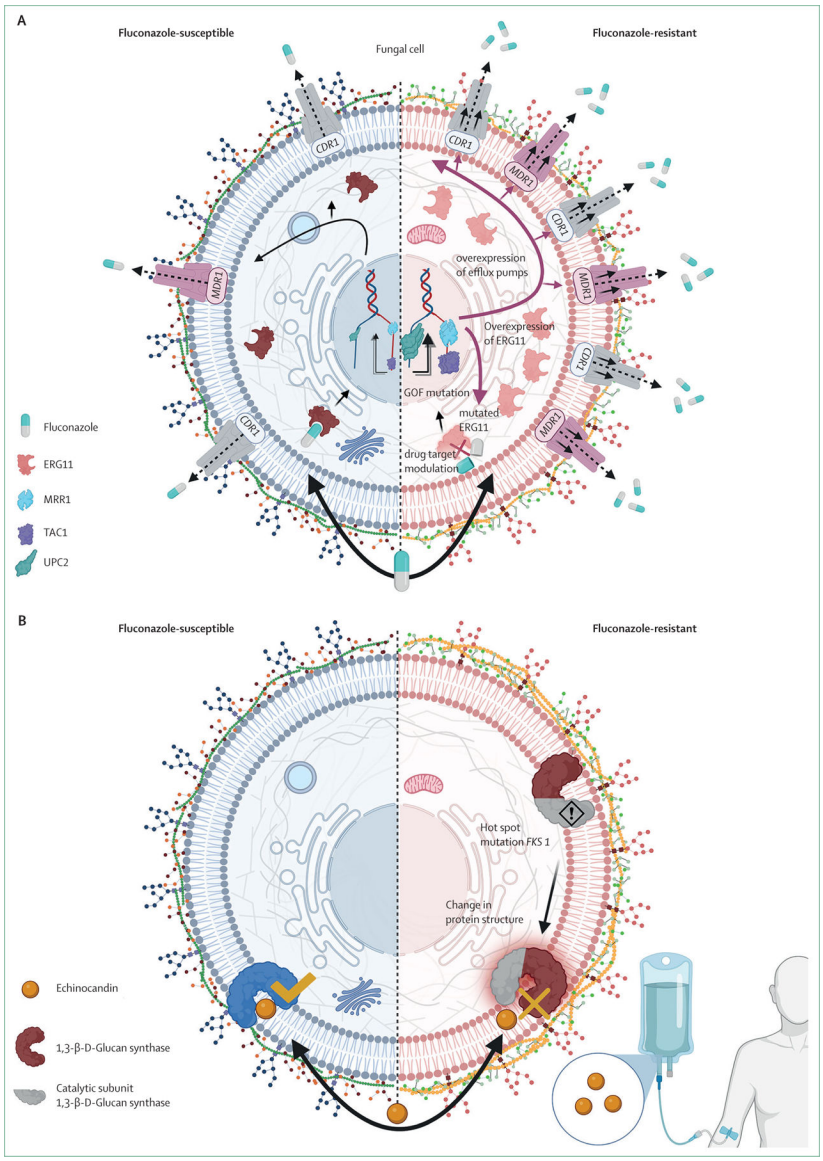

Figure 2: Mechanisms of azole and echinocandin resistance in C parapsilosis

(A) Drug target modulation through acquisition of amino acid substitutions in ERG11—a key enzyme involved in the ergosterol biosynthetic pathway—as well as overexpression of ERG11 and efflux pumps are the main mechanisms underpinning azole resistance in *Candida* species. ERG11 amino acid substitutions, such as Y132F and K143R, are the most prevalent cause of fluconazole resistance in *C parapsilosis*. (B) Echinocandin-resistant *C parapsilosis* isolates mostly harbour mutations in short stretches of the catalytic subunit of the β-glucan synthase, known as hot-spots of *FKS1*. !=presence of a mutation in the catalytic subunit.

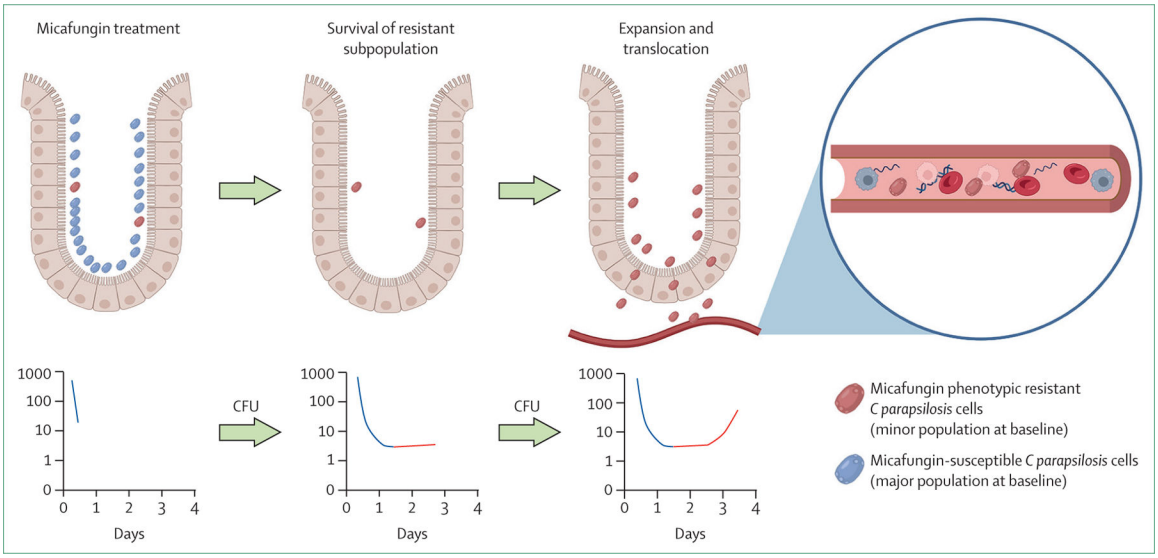

Figure 3: Heteroresistant C parapsilosis isolates are a small subpopulation that tolerate high concentrations of echinocandins.

Echinocandin heteroresistance is a novel concept describing a phenotypically resistant subpopulation that could actively proliferate at a high concentration of a drug and potentially lead to prophylaxis inefficacy. During echinocandin treatment, the susceptible, clonal kins perished, whereas the phenotypically resistant cells survived, replicated, translocated to deeper tissues, and caused systemic infection. The overgrowth of the fungus in the colonisation sites in tandem with virulence and host-related attributes might result in translocation into deeper tissues and result in the failure of antifungal prophylaxis (adapted from references 27 and 38). CFU=colony forming unit.