AR Vaishnavi et al /J. Pharm. Sci. & Res. Vol. 13(11), 2021, 653-660

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Fungal Resistance – An Overview

AR Vaishnavi [1], N Venkateswaramurthy [1*], R Sambath Kumar [2]

Department of Pharmacy Practice[1],
Department of Pharmaceutics [2],
J.K.K.Nattraja College of Pharmacy, Kumarapalayam-638183, Tamil Nadu, India.

* venkateswaramurthy.n@jkkn.ac.in

Abstract:

The widespread uses of triazoles in the early 1990s.Resistance are in both Candida and Aspergillus species has become a global problem. Reports of non-Candida species are been resistant to azoles and to the multidrug-resistant Candida species, such as the Candida glabrata or, lately, Candida auris, these are all increasing with the alarming frequency. Diagnosis of candidiasis has been usually based on the isolation of growing fungus in the blood cultures or tissue biopsy specimens. The sensitivity of the blood cultures for detecting the candidiasis is low, so several days have been required for the development. The usage of molecular beacon probes from reverse-transcribed mRNA of important drug-resistance genes such as ERG11, MDR1, CDR1, and CDR2 has been realised as a nucleic-based detection approach for antifungal resistance in Candida species. Current antifungal treatments are very much limited in their capacity to treat infections, especially in those systemic infections and also no advancements in antifungal therapies were developed recently. The researchers are working to understand the biology of fungal microorganisms in vitro and in vivo. Novel therapeutics based on this interaction could be employed alone or in conjunction with existing antifungal medications. There are various types of pathogenic fungi that have reduced susceptibility to antifungal drugs or have outright resistance to them. Several new antifungals are now being developed, which will be more beneficial than current medications in terms of overcoming antifungal resistance as well as avoiding side effects and drug interactions associated with currently available agents.

Key Words: Antifungal, Candida, Global problem, Resistance.

1. INTRODUCTION:

The skin is the body's outermost layer and the integumentary system's largest organ because it would interface with the environment and also it plays an important role in the protection of the body against the pathogens and also in other environmental conditions. Now-a-days skin diseases are very common among the people, normally 10-15% of the general practioner are work is with the skin disease and it is the second most common cause of loss of performance. [1]

Among the Immunocompromised individual fungal infections are termed to be the major problem. [2] These are all superficial and it can also turn into systemic infections as the disease incidence prolongs. [3] Among the different mycotic infections caused by these opportunistic fungi, candidiasis, infections caused by candida would be the life threatening due to its higher worldwide phenomenon. [4]

Polyenes (amphotericin B), azoles (fluconazole, itraconazole, posaconazole, voriconazole, and isavuconazole), echinocandins (caspofungin, micafungin, and anidulafungin), allylamines (terbinafine), and antimetabolites (flucytosine) are the five major classes of antifungal drugs now in use. [5] Polyene achieve the fungicidal activity by binding to the ergosterol in cell membrane, it would be resulted in the increased permeability and leakage of intracellular components is also a problem., which would be subsequently lead to the cell death also. Similar to these polyenes azoles also targets the ergosterol to achieve the fungicidal activity, [6] by blocking the enzyme lanosterol14-demethylase, they selectively inhibit ergosterol production. Azole's are been the first line therapy due to potent fungicidal activity and also a broad spectrum of coverage. In addition to the ergosterol the other potent antifungal target is 1, 3-β-d-glucan, an important component of fungal cell walls. Echinocandins inhibit 1,3-β-d-glucan synthesis to weaken fungal cell walls and trigger cell lysis. Allylamines interfere with ergosterol synthesis by the inhibition of squalene epoxidase. Terbinafine is a topical antifungal drug that is extensively used in the treatment of dermatophytes and moulds in the salvage context, because to its efficacy and lower adverse effect profile than other antifungal agents. [7] Flucytosine, the final drug of therapeutic significance, is a pyrimidine analogue that acts by selectively interfering with fungal nucleic acid synthesis. [8] However, because of the quick development of resistance, monotherapy is rarely used, and it is mostly used as part of a combination therapy for cryptococcal meningitis, urinary candidiasis, or chromoblastomycosis management.

Antifungal resistance is an evolutionary system primarily based totally on herbal choice of organisms that enchance their capacity to continue to exist and develop withinside the presence of drug. The evolution of resistance in opposition to antimicrobial sellers is ubiquitous in nature and microbes evolve diverse techniques to fight the movement of drugs. [9]

In the tough treatment condition, the prevalence of drug-resistant fungal infections is increasing, which is aggravating. Many of the fungal infections are been having the treatability issues such as the toxicity which is especially for the patients with the other underlying infections (e.g. HIV). Candida auris, a drug-resistant Candida species, is one of the most common causes of invasive fungal infections, with rising resistance to fluconazole, amphotericin B, and voriconazole, as well as emerging caspofungin resistance. [10]

This will lead to the more difficult to treat the fungal infections, treatment failures, longer hospital stays and also much more expensive treatment options. The objective of this study is to know the overview of fungal resistance.

2. EPIDEMIOLOGY:

The introduction and widespread use of triazoles in the early 1990s Resistance are in both Candida and Aspergillus species has become a global problem. [11] Reports of non-Candida species are been resistant to azoles and to the multidrug-resistant Candida species, such as the Candida glabrata [12] or, lately, Candida auris [13], these are all increasing with the alarming frequency. In addition tothe emergence of the Aspergillus species in which they are resistant to triazoles is particularly concerning it because the azoles are theonly oral treatment option for the prophylaxis and long-term treatment of the invasive fungal infection. [14] Iatrogenic selection of the resistance for both the Candida and Aspergillus in the past was typically restricted to the patients who received prolonged treatment courses in the setting of poor source control or persistent severe immunosuppression.[15, 16] Azole-resistant aspergillosisalso been reported on 4 continents [17] and also been hypothesized to be driven by agricultural use of azoles. [14] It would create an different problems because of the need for complex containment strategies that is involved in regulating the agricultural use of the fungicidal agents. Not all the patients with immunosuppression have been similar to the risk for the resistant fungal infections and the type of such infections in which they acquire. Some of the example are, First, patient with chronic granulomatous disease and severe inherent defects in neutrophil function are very rarely develop the resistant mold infection mucormycosis, and the amphotericin B–resistant organism Aspergillus nidulans is a typical pathogen only in these type of patients [18] and also in the other hand patients with other defects in neutrophil function, such as those receiving high doses of corticosteroids for the treatment of chronic graft versus host disease following allogeneic stem cell transplantation, develop this infection, especially if they are hyperglycemic and have been exposed to the antifungal agents that lack Mucorales activity. [19]

3. MECHANISMS OF CLASSIFICATION OF DRUGS INVOLVED IN ANTIFUNGAL RESISTANCE:

3.1 Azoles:

Antifungals based on azoles are one of the most commonly utilised classes in antifungal therapy. The following are the four major mechanisms of azole resistance: (i) Increased drug efflux, (ii) Target Mutation, (iii) Target Expression Deregulation, and (iv) Changes in Ergosterol Biosynthesis Pathway [20]

Increase of drug efflux:

The activation of membrane-associated efflux pumps expels the drug from the cell, lowering the intracellular concentration and leaving a little amount of drug at the action site. [21] The ATP-Binding Cassette (ABC) superfamily and the Major Facilitator Superfamily are two drug efflux mechanisms involved in the clearance of azoles from the cytoplasm of fungi (MFS). [20] Azole resistance is caused by overexpression of the genes that code for transport proteins. CDR1 and CDR2 are the two key ABC transporters genes implicated in azole resistance in Candida albicans; [22] CgCDR1, CgCDR2, and CgSNQ2 in Candida glabrata; and CnAFR1 in Candida neoformans. [23]In C.krusei, the ABC transporters are encoded by two homologous genes, ABC1 and ABC2. In cultures exposed to imidazole, the ABC1 gene is increased, and it is also involved in innate azole resistance. [24] MFS transporters for FLZ are encoded by the MDR1 gene in C. albicans. As a result, transcription of this gene is not linked to azole cross-resistance, but overexpression of the CDR genes can make a cell resistant to a variety of azoles. MDR homologues (CdMDR1 and CtMDR1) have been found to be elevated in azole-resistant C. dubliniensis and C. tropicalis strains, respectively. FLZ resistance has been linked to overexpression of MDR1 and CRD1 in C. parapsilosis, which is caused by concurrent overexpression of MRR1 in conjunction with the mutation. [25]

The efflux pumps have been connected to azole resistance in other fungi. In C. neoformans, overexpression of the AFR1 gene (ABC family) can increase FLZ resistance. Resistance to FLZ and other azoles can also be conferred by Afr2p (ABC family) and Mdr1p (MFS family) from C. neoformans and C. gattii. [24, 26] and Overexpression of Afr1 (ABC family) and mdr3 and mdr4 (MFS family) in Aspergillus spp. has been identified as a resistance mechanism to azoles, particularly itraconazole (ITZ). [27] Increased expression of the ABC transporters genes TruMDR1 and TruMDR2 in T. rubrum has been found in response to the presence of azoles, and appears to be the principal mechanism of resistance in dermatophytes. [24]

Target mutation:

The ERG11 gene has been encoded for the enzyme lanosterol 14ademethylase in yeasts, which is the target enzyme for azoles. Therefore point mutations in these genes alter the azole-binding site, in which it is resulting in reduced affinity or inability to bind drug enzyme. [21] In Aspergillus fumigatus, CYP51A encodes the lanosterol 14a-demethylase and mutations of this gene are the most important step in azole resistance of this species. More than 30 substitution amino acid mutations have been identified in that codons 54 and 220 of CYP51A are the most common.[23] Reduced susceptibility to FLZ and voriconazole has also been linked to a Y136F substitution in Histoplasma capsulatum's CYP51Ap (VOZ).[24]

Target expression deregulation:

Candida spp. thrive in the presence of an azole agent. Overexpression of the ERG11 gene [20] can also create a feedback mechanism in response to ergosterol deficiency, leading in enhanced production of lanosterol 14a-demethylase. A rise in target abundance necessitates the use of more antifungal drugs to counteract the resistance-inducing inhibition. [2]

Ergosterol biosynthesis pathway alteration:

In addition to the mutation of the gene encoding the lanosterol 14a-demethylase enzyme, the mechanism of resistance also alters the other enzymes from the same

biosynthetic mechanism. [21] As a result of azole exposure, fungal membrane ergosterol levels drop and the fungal growth inhibitor 14amethyl-3, 6-diol accumulates. The ERG3 gene mutation hinders the production of 14a-methyl-3, 6-diol from 14a-methyl-fecosterol and accumulates precursors that can replace the cellular ergosterol. These two elements will result in functioning membranes and contribute to Candida spp. resistance development. The ERG3 mutations are then linked to polyene cross-resistance, which is thought to be mediated by ergosterol depletion in the target. [20] Furthermore, mutations in other ergosterol production genes, such as ERG2, ERG6, and ERG24, may be connected to lower azole susceptibility. [24]

3.2 Echinocandins:

The echinocandins, lipopeptides act on the fungal cell walls by specific inhibition of the 1,3-β-Dglucan synthase, which is responsible for the biosynthesis of β-1,3-glucan, the key fungal cell wall component.[28] The enzyme consists of three FKS subunits, called FKS1, FKS2, and FKS3.[29] The resistance is been seen often acquired during therapy by modifying amino acid residues in the FKS1 and FKS2 subunits of β-1, 3-glucan synthase.[30, 31] The function of the FKS3subunit will be remains unclear.[32] The mutations in FKS1 and FKS2 that code for the catalytic subunit genes can be amino acid substitutions that increase MIC levels accompanied by a dramatic reduction inglucansynthase activity.[33] Phe641, Pro649, and Arg1361 (C. albicans homolog) mutations in the FKS1 gene, for example, have been observed. [34] The Ser641 and Ser645 mutations in the FKS2 subunit significantly limit enzyme activity, resulting in a marked resistance phenotype. [35] The FKS1 and FKS2 amino acid residue alterations differed depending on the fungus species, as well as the resistance to echinocandins. [36] Inhibition of 1, 3-glucan synthase echinocandin causes cell wall defects, which causes cellular stress and several genes are expressed to adapt to this stress condition. Protein kinase C (PKC) regulates the manufacture of different carbohydrates in the cell wall, HOG (high-osmolarity glycerol) [37, 38] and chitin biosynthesis, which has been linked to echinocandin resistance. [39] Overall, this salvage mechanism has been found to aid the fungus in adaptive cell wall remodelling, allowing cells to survive even when exposed to higher echinocandin levels. Dimorphic fungi have the natural resistance to the echinocandins during the pathogenic phase, but the resistance mechanisms of the β-glucan synthase inhibitors are currently not known. [40, 41]

3.3 Polyenes:

Resistance to polyenes also beendeveloped slowly over time, because the interaction of amphotericin B with the plasma membrane is complex, and multiple changes may be required to prevent disruption of the cell membrane. Mechanisms of resistance to the polyenes includes the alterations in the membrane sterols, defense mechanisms which are against oxidative damage, defects in the ergosterol biosynthetic genes, factors such as the fatty acid composition of the cell membrane, and alterations in the sterol to phospholipid ratio. Existing ergosterol structure can be reoriented or hidden – for example, by sequestration within phagocytes – resulting in steric interference between the polyene and the ergosterol.[42] The growth phase of the fungal cell and changes in cell wall structure has been involved in polyene resistance.

4. DIAGNOSIS OF FUNGAL RESISTANCE:

In vitro susceptibility-testing methods are still one of the time consuming and costly. Clinicians need to be fast and reliable analysis of the patient samples to permit the early decisions about initiating the most effective therapy. Diagnosis of candidiasis has been usually based on the isolation of growing fungus in the blood cultures or tissue biopsy specimens. The sensitivity of the blood cultures for detecting the candidiasis is low, especially in early stages of the infections, so several days have been required for the development. In which susceptibility tests have been performed from blood cultures, the delay for resistance diagnosis are even greater. The diagnosis of mold infections often based on the clinical and nonspecific radiographic observations, and confirmed diagnosis requires demonstration of fungal cells from clinical pattern. Only antifungal susceptibility testing can be performed once the fungal culture is available. Molecular strategies for measuring primary and secondary resistance have proven to be faster and more accurate than traditional methods. [43] These procedures may be used instead of traditional methods since they reduce the time it takes for professionals to acquire a diagnosis. The usage of molecular beacon probes from reverse-transcribed mRNA of important drug-resistance genes such as ERG11, MDR1, CDR1, and CDR2 has been realised as a nucleic-based detection approach for antifungal resistance in Candida species. [44] The single-stranded oligonucleotide hybridization probes that make up the stem-and-loop structure are known as molecular beacons. When they are bound to a target, they should undergo a structural change that may be seen using covalently coupled fluorochromes. Overexpression of CDR1, CDR2, and MDR1, as well as four mutations in ERG11, were all linked to the fluconazole-resistant phenotype (MIC64 g/mL) (T229A, Y132F, S405F, G464S). Starting with fungal DNA or patient samples, molecular beacon technology could be applied straight to the identification of gene alterations. In Candida species, where changes in FKS1/FKS2 are restricted to specific areas, similar approaches can be used to discover echinocandin resistance. [45] Microarray technologies and in situ primer extension of mutations with fluorochrome-derived nucleotides are being used to establish further diagnostic platforms for detecting azole resistance in Candida (on-CHIP minisequencing). For each of the known drug-resistance gene mutations, individual probes have been put in the microarrays. A laser scanner reveals fluorochrome incorporations on specified array points. The advantage of this method is that thousands of probes can be printed on the arrays, corresponding to all known mutations from various fungus species. Several reports have documented the diagnosis of antifungal drug resistance in Aspergillus species. Because there are so few

mutations in cyp51A that cause azole resistance, creating a molecular diagnostic tool that is less challenging than Candida species has been a challenge. [46] The initial concept for the multiplex panel of allele-specific probes was to identify particular mutations linked to itraconazole resistance. [47] Molecular beacons belonging to the wild-type cyp51A gene (codon location G54) and seven mutant alleles with positions R54, K54, V54, W54, or Q54 were employed in the assay. The multiplex assay was used to examine 48 A. fumigates isolates, the majority of which had reduced itraconazole susceptibility. All of the strains studied had their cyp51A allelic identities for codon 54 established, and mutations affecting G54 in 23 strains were discovered. A second sequencing of cyp51A alleles validated the identified mutation in the study. The created multiplex real-time PCR with molecular beacons was deemed to be the most powerful technology for allele differentiation, and was shown to be suited for quick, high-throughput drug resistance evaluation. It was expanded to include all known cyp51A alleles that confer itraconazole, voriconazole, and posaconazole resistance. [48] Because azole resistance is allele-dependent, the assay's availability could allow for the prediction of specific resistance patterns in specific isolates. The experiment was only carried out on farmed A. fumigatus colonies, although it could theoretically be used to primary specimens as well. The sensitive PCR detection approach was used to measure respiratory fungal burden in bronchoalveolar lavage (BAL) and sputum specimens in a recent report. [49] Cyp51A was further amplified using this approach in a subset of the PCR-positive, culture-negative samples to find a critical mutation linked to triazole resistance. Surprisingly, triazole-resistance mutations (L98H with tandem repeat and M220) were found in 55.1 percent of the culture-negative, PCR-positive samples. When low organism loads of fungus causing infection could not be detected by direct culture, this study indicated that resistance detection can be done from clinical samples. A separate study was developed a protocol which is based on the detection of the cyp51A alleles causing azole resistance but using Taqman probes. The samples in this case came from formalin-fixed and paraffin-embedded tissue slices. A mutation in cyp51A (L98H) and a tandem repeat in the cyp51A promoter were shown to be responsible for resistance to all known azoles, according to the researchers. [50] Both investigations showed that early detection of azole resistance are possible, which has substantial implications for the long-term use of azoles in human antifungal treatment.

5. RISK FACTORS FOR FAILURE OF ANTIFUNGAL THERAPY:

When the antifungal therapy fails, either the primary or acquired drug resistance of the organism has to be appraised. However, there are other factors which relates to both of the drug and then infected host which also play a significant role, and these have to be taken into consideration. When medication is given orally, there is a risk of poor absorption and low serum/tissue drug concentrations, which can lead to treatment failure. When itraconazole pills were given to neutropenic patients, it was reported. Another issue with azole use is lower efficacy due to faster metabolism when used with other medications that stimulate hepatic cytochrome P450 enzymes, such as rifampicin, which is one of the most common examples. The drug's pharmacokinetic characteristic has to be taken into account as well. Amphotericin B has a low penetration of the blood-brain barrier, but 5 Fluorocytosine has a high penetration; this could explain the better outcome of cryptococcal meningitis when these two treatments are taken together rather than amphotericin alone. The immunological status of the infected host is thought to play a role in the outcome of an invasive fungal infection. CD4' lymphocyte count of 50 cells/ mm3, indicating advanced AIDS, recurrent bouts of OPC, and lengthy past exposure to azoles are all risk factors for acquired fluconazole resistance in HIV patients. [51] In the case of neutropenic individuals, invasive aspergillosis frequently fails to respond to antifungal medications, despite the fact that bone marrow loss persists. [52] Antifungal medicines are frequently ineffective in patients who have recovered from neutropenia and develop the unusual disease chronic hepatic candidiasis, but they may be effective when paired with immunotherapeutic treatments. [53] The presence of vascular catheters is now well acknowledged as a key risk factor for candidemia development. Their presence also raises the risk of infection recurrence after effective therapy. [54] The formation of Candida biofilms coating the catheter's lumen appears to allow the organism to persist and withstand antifungal medication action. [55]

6. STRATEGIES TO CONTROL FUNGAL INFECTIONS:

Current antifungal treatments arevery much limited in their capacity to treat infections, especially in those systemic infections and also no advancements in antifungal therapies were developed recently. New therapies are much needed against the pathogenic fungi. Researchers hope to find new antifungal medications by evaluating existing medical chemicals, compounds from natural sources such as plants, sea, and microbes, or by screening chemical compound libraries in a systematic manner. The researchers are working to understand the biology of fungal microorganisms in vitro and in vivo. For all fungal diseases, host-fungal interactions are also important. Novel therapeutics based on this interaction could be employed alone or in conjunction with existing antifungal medications. The development of antifungal medication resistance may be influenced by such combinations.

6.1 Development of New Antifungal Active Compounds:

Natural compounds (NP) or natural bioactive compounds obtained from plants, other microbes, or marine species have been studied extensively for their antifungal activities. [56, 57] If you're looking for a unique way to express yourself other chemicals are evaluated blindly for antifungal agents, while these compounds are researched because their recognised triggering mechanisms are critical for fungi. Despite some promising results, none of

this research has resulted in a chemical eligible for clinical trials. Other investigations focused on in vitro screenings of a number of medications currently in clinical use for their ability to potentiate the antifungal activity of the fungistatic agent fluconazole (FLC) on Candida albicans. Several drugs, such as inhibitors of the calcineurin [58] or Tor pathways, [59] efflux pump inhibitors (derived compounds of milbemycin), [60] and more recently, antibodies against the heat-shock 90 protein, were discovered as a result of this (HSP90). [61] Inhibitors of the calcineurin pathway, in particular, were found to be fully active in vivo in the potentiation of fluconazole and to result in a significant reduction in fungus virulence. [62, 63] Systematic screening of chemical compound libraries was also carried out, primarily by industrial laboratories in the search for novel antifungal chemicals. The discovery of a replacement glucan synthase inhibitor effective against Candida albicans and Candida glabrata came as a result of high throughput screening of the heritage Schering-Plough chemical library. [64] Reverse genetic assays were utilised in which C. albicans heterozygous deletion or transposon disruption mutants were evaluated for growth while being treated with various chemical substances. [65] This method allowed researchers to find antifungal drugs as well as genes related to the compounds' mechanisms of action.

Screening in chemical libraries was used to determine the survivability of drug-treated Caenorhabditiselegans infected with Candida albicans. Compounds can be evaluated for antifungal activity and host toxicity at the same time, overcoming one of the present antimicrobial discovery's biggest challenges. Using this unique C. elegans system, a pilot screen for antifungal compounds discovered 15 compounds that extended the survival of worms infected with the medically significant human disease Candida albicans. In a mouse model of systemic candidiasis, one of these compounds, caffeic acid phenethyl ester (CAPE), demonstrated effective antifungal action, as well as in vitro efficacy against numerous other fungal species. [66] Furthermore, using this whole-animal approach, researchers may be able to identify chemicals that influence immune responses and/or fungal virulence factors that are only expressed during infection.

6.2 Genome:

The development of medications that exploit features unique to fungus, which can be difficult given that the organisms are eukaryotic and share many common biological processes, is a major challenge for antifungal therapy. Drug development could focus on genes that are necessary for fungal survival. Some research groups have investigated the essentiality of C. albicans and Aspergillus fumigatus genes using the GRACE (gene replacement and conditional expression) or CPR (conditional promoter replacement) technologies. [67] In C. albicans, one study discovered 567 important genes. [68] Another study evaluated 54 A. fumigatus genes for ortholog functions and essentiality in Candida albicans and Saccharomyces cerevisiae, with 35 of them being designated as essential in A. fumigatus. The researchers were prepared to demonstrate that while the ERG11 gene family (CYP51A and ERG11B) is vital during a fumigatus, the individual genes are not. These investigations will give intriguing and fully meaningful data for antifungal drug design, and will improve on prior in silico analyses that only identified 61 percent of homologous genes reported in the genes detected in the Roemer et al. analysis when utilising S. cerevisiae data. [67] The diploid state of the genome is a fundamental impediment to mutant collection development. As a result, several of the collections include mutants with heterozygous deletion [69] or transposon disruption. The random insertion of the Tn7 transposon to a UAU cassette was supported in other collections by homozygous transposon disruption mutants. [70] These collections were the first to be limited to C. albicans transcription factors, [71] but they are now being expanded to include the whole genome. [72] The deletion mutants built with PCR-generated deletion cassettes, with two distinct markers for each allele in the case of C. albicans, are included in the other collections. [73] Such collections are now being constructed for the C. glabrata.

A deeper understanding of the changes that occur in fungi that have been exposed to antifungal therapies, as well as the relationship that has established between the fungus and its host during the infectious process. Such knowledge will improve real therapy in order to avoid the development of resistance, or it may allow for the manipulation of the host-fungus equilibrium in order to improve patient recovery. First, some writers aim to better understand pharmacological mechanisms of action and/or uncover synergistic effects by treating strains with already known antifungal medications and analysing it for example, growth modification and subsequent transcriptional rewiring. [72] The transcription factor Cas5-encoding gene was discovered to be implicated in the response to caspofungin. Other research has found that deleting AGE3, which encodes an ADP-ribosylation factor GTPase activating effector protein, eliminates fluconazole tolerance in Candida albicans. Brefeldin A, an inhibitor of ADP-ribosylation factor, has a synergistic impact with other medicines for both Candida albicans and Aspergillus niger. Finally, Homann et al. evaluated a collection of 143 transcription factor mutants under 55 different circumstances, including fluconazole exposure and 5 Flucytosine exposure, and they conclude in their analysis that nearly a quarter of the knockout strains affected sensitivity to commonly used antifungal drugs.[73] Other research have improved our understanding of the biology of fungal species. Mutant collections were exposed to a wide range of environmental variables for this study, including pH, salt concentration, carbon sources, oxidative conditions, temperature, and the availability of critical components like metals (iron, copper, zinc, etc.). [74] Gaining a better understanding of the connection between the fungus and the host during infections may provide more knowledge that might help enhance antifungal treatment. Researchers screened the colonisation properties of mutants directly into the hosts to analyse the cross-talk that occurs between the fungus and the host during the infectious process. In one study, researchers looked at the in vivo proliferation profile of 1201 gene

knockout mutants of Cryptococcus neoformans in the mouse lung and found 40 infectivity mutants. [75] The gene deletions in these mutants had never been studied before, and they did not demonstrate any defects in virulence-related features (polysaccharide capsule formation, melanization, and growth at body temperature). At least four further experiments involving C. albicans mutations have been conducted. Two of them were invertebrate host models such as C. elegans or D. melanogaster that had been wipped out. It's worth noting that the Cas5/ mutant, which has been proven to be crucial for caspofungin response, was shown to be less virulent in both invertebrate infection models. Finally, this transcription factor was shown to be important for cell membrane integrity, and its role in virulence was verified in a mouse intravenous infection model. [76] Two other investigations used pools of previously tagged mutants to screen collections of C. albicans mutants directly in mice. One collection was limited to Zn2-Cys6 transcription factors (TF) mutants, whereas the other included mutants impacting roughly 11% of the overall C. albicans genome, regardless of gene class. In both cases, mutants were tested for virulence traits such as filamentation and proliferative ability, as well as the ability to grow at 42°C, at high and low pH, and in oxidative conditions. [77] Noble et al. discovered 115 mutants, out of which 674 were found to have reduced infectivity but normal morphological switching and proliferation. [78] They discovered that glycolipid and glucosylceramide are the first small compounds generated by Candida albicans that are necessary for virulence. Within the 77 Zn2-Cys6 TF mutants investigated, Vandeputte et al. discovered two. In their pool test, these mutants showed reduced infectivity, which was validated in independent single strain infections of mice ZCF13 and ZCF18. [79] Previously, neither of the genes had been identified. In contrast to ZCF13, which developed normally at blood heat but somewhat less at 42C, ZCF18 revealed a minor growth problem. The ZCF13 mutant had an abnormal morphology, producing filamentous colonies on YPD medium at 35°C and having high invasion ability. The elimination of ZCF18 resulted in a slight increase in colony wrinkling. Both genes are now being investigated further. Unfortunately, despite the fact that these approaches have shown promise, no new compound and/or new target has been chosen for further study.

7. CONCLUSION:

Clinicians are currently dealing with a number of new antifungal resistance concerns. These include azole and echinocandin resistance in various non-C.albicans species, as well as azole resistance in A. fumigatus, which may emerge as a result of clinical or environmental exposure to these compounds. There are various types of pathogenic fungi that have reduced susceptibility to antifungal drugs or have outright resistance to them. Several new antifungals are now being developed, which will be more beneficial than current medications in terms of overcoming antifungal resistance as well as avoiding side effects and drug interactions associated with currently available agents. To determine if these medicines will be successful in overcoming microbiologic and clinical failure in the context of antifungal resistance, more preclinical and clinical research is required.

Conflicts of Interest: Authors declare that they have no conflicts of interest.

Funding statement: This review did not receive any funding.

REFERENCE:

1. Dixit, H., Sharma, A., Chandel, V., *et al.,* Ayurvedic methods of diagnosis and management of dadrukushta W.S.R. to fungal infection. *Int J Health Sci Res*. 2020, 10(3), 114-120.
2. Shapiro, R.S., Robbins, N., Cowen, L.E., Regulatory circuitry governing fungal development, drug resistance, and disease. *MicrobiolMol Biol*.2011, 75, 213–267.
3. Cannon, R.D., Lamping, E., Holmes, A.R., *et al.,* Efflux-mediated antifungal drug resistance. *Clin Microbiol*.2009, 22, 291–321.
4. Lopez, R., Candidosis a new challenge. *Clin Dermatol*.2010, 28,178–184.
5. McCarthy, M.W., Kontoyiannis, D.P., Cornely, O.A., *et al.,* Novel agents and drug targets to meet the challenges of resistant fungi. *J. Infect. Dis.* 2017, 216, 474–483.
6. Van Daele, R., Spriet, I., Wauters, J., *et al.*, Antifungal drugs: What brings the future? *Med. Mycol*. 2019, 57, 328–343.
7. Balfour, J.A., Faulds, D., Drug Evaluation Terbinafine a Review of Its Pharmacodynamic and Pharmacokinetic Properties, and Therapeutic Potential in Superficial Mycoses. *Drugs*.1992, 43, 259–284.
8. Perfect, J.R., The antifungal pipeline. *Drug Discov*. 2017, 16, 603–616.
9. Peman, J., Canton, E., Espinel, A., Antifungal drug resistance mechanisms. *Anti Infect. Ther*. 2009, 7, 453–460.
10. World Health Organisation. Microbial resistance. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance.
11. Kontoyiannis, D.P., Lewis, R.E., Antifungal drug resistance of pathogenic fungi. *Lancet*. 2002, 359, 1135–1144.
12. Healey, K.R., Zhao, Y., Perez, W.B., *et al.,* Prevalent mutator genotype identified in fungal pathogen Candida glabrata promotes multi-drug resistance. *Nat Commun*. 2016, 7, 11128.
13. Lockhart, S.R., Etienne, K.A., Vallabhaneni, S., *et al.,* Simultaneous emergence of multidrug-resistant Candida auris on 3 continents confirmed by whole-genome sequencing and epidemiological analyses. *Clin Infect Dis*. 2017, 64, 134–140.
14. Verweij, P.E., Chowdhary, A., Melchers, W.J., Azole resistance in Aspergillus fumigatus: can we retain the clinical use of mold-active antifungal azoles? *Clin Infect Dis*. 2016, 62, 362–368.
15. Arendrup, M.C., Melchers, W.J., Verweij, P.E., Azole resistance of Aspergillus fumigatus in immunocompromised patients with invasive Aspergillosis. *Emerg Infect Dis*. 2016, 22,158–159.
16. Jung, D.S., Farmakiotis, D., Jiang, Y., *et al.*, Uncommon candida species fungemia among cancer patients, Houston, Texas, USA. *Emerg Infect Dis*. 2015, 21, 1942–1950.
17. Linden, J.W., Arendrup, M.C., Warris, A., *et al.,* Prospective multicenter international surveillance of azole resistance in Aspergillus fumigatus. *Emerg Infect Dis*. 2015, 21, 1041–1044.
18. Bauer, I., Gross, S., Merschak, P., *et al.*, RcLS2F - A Novel Fungal Class 1 KDAC Co-repressor Complex in Aspergillus nidulans. *Front Microbiol*. 2020, 11, 43.
19. Kontoyiannis, D.P., Lionakis, M.S., Lewis, R.E., *et al.*, Zygomycosis in a tertiary-care cancer center in the era of Aspergillus-active antifungal therapy: a case-control observational study of 27 recent cases. *J Infect Dis*. 2005, 191, 1350–1360.
20. Tobudic, S., Kratzer, C., Presterl, E., Azole-resistant Candida spp.-emerging pathogens? *Mycoses*.2012, 5, 24–32.
21. Pfaller, M., Antifungal drug resistance: mechanisms, epidemiology, and consequences for treatment. The American Journal of Medicine *Am J Med*.2012, 12, 3–13.

22. Sanglard, D., Emerging threats in antifungal-resistant fungal pathogens. *Front Med*.2016, 3, 1–10.
23. Cowen, L.E., Sanglard, D., Howard, S.J., *et al.,* Mechanisms of antifungal drug resistance. *Cold Spring HarbPerspect Med*.2015, 5(7), 1-24.
24. Scorzoni, L., Silva, A.P., Marco, C.M., *et al.,* Antifungal therapy: new advances in the understanding and treatment of mycosis. *Front Microbiol*.2017, 8, 1–23.
25. Zhang, L., Xiao, M., Watts, M.R., *et al.,* Development of fluconazole resistance in a series of Candida parapsilosis isolates from a persistent candidemia patient with prolonged antifungal therapy. *BMC Infect Dis*.2015, 15, 340.
26. Basso, L.R., Gast, C.E., Bruzual, I., Identification and properties of plasma membrane azole efflux pumps from the pathogenic fungi Cryptococcus gattii and Cryptococcus neoformans. *J AntimicrobChemother*. 2015, 70, 1396–1407.
27. Vandeputte, P., Ferrari, S., Coste, A.T., Antifungal Resistance and New Strategies to Control Fungal Infections. *Int J Microbiol*. 2012, 7(1), 1–26.
28. Denning, D.W., Echinocandin antifungal drugs. *Lancet.*2003, 362 (9390), 1142–1151.
29. Johnson, D.S., Perlin, T.D., Fks1 and Fks2 are functionally redundant but differentially regulated in Candida glabrata: implications for echinocandin resistance. *Antimicrob. Agents Chemother*. 2012, 56(12), 6304–6309.
30. Thompson, G.R., Wiederhold, N.P., Vallor, A.C., *et al.,* Development of caspofungin resistance following prolonged therapy for invasive candidiasis secondary to Candida glabrata infection. *Antimicrob. Agents Chemother.* 2008, 52(10), 3783–3785.
31. Garnaud, C., Botterel, F., Sertour, N., *et al.,* Next-generation sequencing offers new insights into the resistance of Candida spp. to echinocandins and azoles. *J Antimicrob Chemother J Antimicrob Chemother*. 2015, 70(9), 2556–2565.
32. Perlin, D.S., Current perspectives on echinocandin class drugs. *Future Microbiol*.2011, 6(4), 441–457.
33. Shields, R.K., Nguyen, M.H., Press, E.G., *et al.,* Rate of FKS mutations among consecutive Candida isolates causing bloodstream infection. *Antimicrob. Agents Chemother*. 2015, 59(12), 7465–7470.
34. Katiyar, S.K., Edlind, T.D., Role for Fks1 in the intrinsic echinocandin resistance of Fusariumsolani as evidenced by hybrid expression in Saccharomyces cerevisiae. *Antimicrob. Agents Chemother*.. 2009, 53(5), 1772–1778.
35. Effron, G., Lee, S., Park, S., *et al.,* Effect of Candida glabrata FKS1 and FKS2 mutations on echinocandin sensitivity and kinetics of 1,3-beta-D-glucan synthase: implication for the existing susceptibility breakpoint. *Antimicrob. Agents Chemother*.. 2009, 53(9), 3690–3699.
36. Pasquale, T., Tomada, J.R., Ghannoun, M., *et al.,* Emergence of Candida tropicalis resistant to caspofungin. *J Antimicrob Chemother*.2008, 61(1), 219.
37. Garcia, R., Botet, J., Rodriguez-Pena J.M., *et al.,* Genomic profiling of fungal cell wall-interfering compounds: identification of a common gene signature. *BMC Genomics.* 2015, 16, 683.
38. Perlin, D.S., Mechanisms of echinocandin antifungal drug resistance. *Ann. N. Y. Acad. Sci*. 2015, 1354, 1–11.
39. Walker, L.A., Gow, N.A., Munro, C.A., Elevated chitin content reduces the susceptibility of Candida species to caspofungin. *Antimicrob Agents Chemother*. 2013, 57(1), 146–154.
40. Ueno, K., Namiki, Y., Mitani, H., *et al.,* Differential cell wall remodeling of two chitin synthase deletants Deltachs3A and Deltachs3B in the pathogenic yeast Candida glabrata. *FEMS Yeast Res.* 2011, 11(5), 398–407.
41. Goughenour, K.D., Rappleye, C.A., Antifungal therapeutics for dimorphic fungal pathogens. *Virulence.*2017, 8(2), 211–221.
42. Shaughnessy, M.O., Caron, A., Thomas, J., Amphotericin B: Polyene Resistance Mechanisms. *Microbial Drug Resist*. 2009, 25, 295-305.
43. Perlin, D.S., Antifungal drug resistance: do molecular methods provide a way forward? *Curr Opin Infect Dis*. 2009, 22, 568-573.
44. Park, S., Perlin, D., Establishing surrogate markers for fluconazole resistance in Candida albicans. *Microb Drug Resist*. 2005, 11, 232-238.
45. Balashov, S.V., Park, S., Perlin, D.S., Assessing resistance to the echinocandin antifungal drug caspofungin in Candida albicans by profiling mutations in FKS1. *Antimicrob Agents Chemother*. 2006, 50, 2058-2063.
46. Perlin, D.S., Zhao, Y., Molecular diagnostic platforms for detecting Aspergillus. *Med Mycol*. 2009, 47(1), 223-232.
47. Balashov, S.V., Gardiner, R., Park, S., *et al.,* Rapid, high-throughput, multiplex, real-time PCR for identification of mutations in the cyp51A gene of Aspergillus fumigatus that confer resistance to itraconazole. *J ClinMicrobiol*. 2005, 43, 214-222.
48. Effron, G., Dilger, A., Alcazar-Fuoli, L., *et al.,* Rapid detection of triazole antifungal resistance in Aspergillus fumigatus. *J ClinMicrobiol*. 2008, 46, 1200-1206.
49. Denning, D.W., Park, S., Lass C., *et al.,* High-frequency triazole resistance found in nonculturable Aspergillus fumigatus from lungs of patients with chronic fungal disease. *Clin Infect Dis*. 2011, 52, 1123-1129.
50. Linden, J.W., Snelders, E., Arends, J.P., *et al.,* Rapid diagnosis of azole-resistant aspergillosis by direct PCR using tissue specimens. *J ClinMicrobiol*. 2010, 48, 1478-1480.
51. Rex, J.H., Rinaldi, M.G., Pfaller, M.A., Resistance of Candida species to fluconazole. *Antimicrob Agents Chemother*. 1995, 39, 1-8.
52. Maenza, J.R., Keruly, J.C., Moore, R.D., *et al.,* Risk factors for fluconazole resistant candidiasis in HIV infected patients. *J Infect Dis*. 1996, 173, 219-225.
53. Poynton, C.H., Barnes, R.A., Rees, J., Interferon gamma and granulocyte-macrophage colony stimulating factor for the treatment of hepatospleniccandidosis in patients with acute leukemia. *Clin Infect Dis*. 1998, 26, 239-240.
54. Rex, J.H., Bennett, J.E., Sugar, A.M., *et al.,* Intravascular catheter exchange and duration of candidemia. *Clin Infect Dis*. 1995, 21, 994-996.
55. Baillie, G.S., Douglas, L.J., Role of dimorphism in the development of Can&daalbicuns biofilms. *J Med Microbial.* 1999, 48, 671-679.
56. Santo, R., Natural products as antifungal agents against clinically relevant pathogens. *Natural Product Reports*.2010, 27(7), 1084-1098.
57. Mayer, A.M., Rodguez, A.D., Fusetani, N., Marine pharmacology in 2007-8: marine compounds with antibacterial, anticoagulant, antifungal, antiinflammatory, antimalarial, antiprotozoal, antituberculosis, and antiviral activities; affecting the immune and nervous system, and other miscellaneous mechanisms of action. Comparative Biochemistry and Physiology. 2011, 153(2), 191–222.
58. Marchetti, O., Entenza, J.M., Sanglard, D., *et al.,* Fluconazole plus cyclosporine: a fungicidaetal.l combination effective against experimental due to Candida albicans. *Antimicrob Agents Chemother*. 2000, 44(11), 2932–2938.
59. Cruz, M.C., Goldstein, A.L., Blankenship, L., *et al.,* Rapamycin and less immunosuppressive analogs are toxic to Candida albicans and Cryptococcus neoformans via FKBP12-dependent inhibition of TOR. *Antimicrob Agents Chemother*. 2001, 45(11), 3162–3170.
60. Cannon, R.D., Lamping, E., Holmes, A.R., *et al.,* Effluxmediated antifungal drug resistance. *Clin Microbiol Rev*. 2009, 22(2), 291–321.
61. Pachl, J., Svododa, P., Jacobs, F., *et al.,* A randomized, blinded, multicenter trial of lipid-associated amphotericin B alone versus in combination with an antibody-based inhibitor of heat shock protein 90 in patients with invasive candidiasis. *Clin Infect Dis.* 2006, 42(10), 1404–1413.
62. Bader, T., Schroppel, K., Bentink, S., *et al.,* Role of calcineurin in stress resistance, morphogenesis, and virulence of a Candida albicans wild type strain. *Infect Immun*. 2006, 74(7), 4366– 4369.
63. Steinbach, W.J., Cramer, R.A., Perfect, B.Z., *et al.,* Calcineurin controls growth, morphology, and pathogenicity in Aspergillus fumigate. *Eukaryotic Cell*. 2006, 5(7), 1091– 1103.
64. Ting, P.C., Kuang, R., Wu, H., *et al.,* The synthesis and structure-activity relationship of pyridazinones as glucan synthase inhibitors. *Bioorganic Med Chem Lett*. 2011, 21(6), 1819–1822.
65. Oh, J., Fung, E., Schlecht, U., *et al.,* Gene annotation and drug target discovery in Candida albicans with a tagged transposon mutant collection. *PLoS Pathogens*. 2010, 6(10), e1001140.
66. Pukkila, R., Holson, E., Wagner, F., Antifungal drug discovery through the study of invertebrate model host. *Curr Med Chem*. 2009, 16(13), 1588–1595.

67. Roemer, T., Jiang, B., Davison, J., *et al.*, Large-scale essential gene identification in Candida albicans and applications to antifungal drug discovery. *Mol Microbiol*. 2003, 50(1), 167–181.
68. Hu, W., Sillaots, S., Lemieux, S., *et al.*, Essential gene identification and drug target prioritization in Aspergillus fumigates. *PLoS Pathogens*. 2007, 3(3), e24.
69. Xu, D., Jiang, B., Ketela, T., *et al.*, Genome-wide fitness test and mechanism-of-action studies of inhibitory compounds in Candida albicans. *PLoS pathogens*. 2007, 3(6), e92.
70. Nobile, C., Mitchell, A., Large-scale gene disruption using the UAU1 cassette. *Methods Mol Biol*. 2009, 499, 175-194.
71. Bruno, V.M., Kalachikov, S., Subaran, R., *et al.*, Control of the C. albicans cell wall damage response by transcriptional regulator Cas5. *PLoS Pathogens.* 2006, 2(3), 204-210.
72. Epp, E., Vanier, G., Harcus, D., *et al.*, Reverse genetics in Candida albicans predicts ARF cycling is essential for drug resistance and virulence. *PLoS Pathogens*. 2010, 6(2), e1000753.
73. Homann, O.R., Dea, J., Noble, S.M., A phenotypic profile of the Candida albicans regulatory network. *PLoS Genetics*. 2009, 5(12), e1000783.
74. Epp, E., Walther, A., Lepine, G., *et al.*, Forward genetics in Candida albicans that reveals the Arp2/3 complex is required for hyphal formation, but not endocytosis. *Mol Microbiol*. 2010, 75(5), 1182–1198.
75. Liu, O.W., Chun, C.D., Chow, E.D., *et al.*, Systematic genetic analysis of virulence in the human fungal pathogen Cryptococcus neoformans. *Cell*. 2008, 135(1), 174–188.
76. Chamilos, G., Nobile, C.J., Bruno, V.J., *et al.*, Candida albicans Cas5, a regulator of cell wall integrity, is required for virulence in murine and toll mutant fly models. *J Infect Dis.* 2009, 200(1), 152–157.
77. Worley, R., Peleg, A.Y., Tampakakis, E., Candida albicans hyphal formation and virulence assessed using a caenorhabditiselegans infection model. *Eukaryotic Cell*. 2009, 8(11), 1750–1758.
78. Vandeputte, P., Ischer, F., Sanglard, D., In vivo systematic analysis of Candida albicans Zn2-Cys6 transcription factors mutants for mice organcolonization. *Plos One*.2011, 6(10), e26962.
79. Noble, S.M., French, S., Kohn, L.A., *et al.*, Systematic screens of a Candida albicans homozygous deletion library decouple morphogenetic switching and pathogenicity. *Nat. Genet*. 2010, 42(7), 590–598.