Non albicans Candida species: A review of epidemiology, pathogenicity and antifungal resistance.

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Abstract

Among various pathogenic fungi, Candida species is the only pathogen capable of causing wide spectrum of clinical manifestations ranging from mucocutaneous overgrowth to disseminated infections. Although Candida albicans is considered as the most pervasive pathogenic species, recent studies from various parts of the world have documented the emergence of non albicans Candida (NAC) spp. The Candida isolates belonging to NAC spp. differ in the terms of epidemiology, pathophysiology and of most important the pattern of susceptibility to a particular antifungal drug. Therefore species identification along with in-vitro susceptibility has became important for 'species directed therapy' and selection of appropriate antifungal therapeutic agent.

Key words: *Candida albicans*, candidiasis, non *albicans Candida*, species identification, susceptibility testing.

Introduction

Infections have always been a feature of human life and continue to be a significant problem for health care professionals across the globe. The last three decades have witnessed a significant rise in the incidence of infections due to fungal pathogens. Although numerous factors are implicated in the increased occurrence of mycotic infections, certain factors like advent of HIV/ AIDS, increased and widespread use of immunosuppressive therapies and broad spectrum and invasive surgical interventions are particularly important. [1]

Among various pathogenic fungi, the members of genus *Candida* are most frequently isolated from human infections. Although *Candida albicans* is considered as the most pervasive pathogenic species, recent studies from various parts of the world have documented the emergence of non *albicans Candida* (NAC) spp. [2]

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The drastic epidemiological shift in trend of candidiasis from *C. albicans* to NAC spp. can be related to widespread empirical use of azole group of antifungal agent for prophylaxis and treatment of mycoses and utilization of commercially available diagnostic kits for identification of pathogenic fungi. [3]

The shift towards NAC spp. is of concern because, the *Candida* isolates belonging to this group often demonstrate innate or acquired or both reduced susceptibility to fluconazole, the most cost-effective and readily available antifungal drug for treatment of candidiasis. [2] In the present review, the epidemiological and clinical features of NAC spp. are addressed together with their virulence factors and antifungal resistance pattern.

Method

For preparation of this review, the relevant research and review articles were retrieved from databases like Pubmed, Scopus and Google Scholar. We performed searches on these databases using 'MeSh' terms like '*Candida* species', 'candidiasis', 'non albicans *Candida* species', 'virulence factors of *Candida*', 'pathogenicity of *Candida*', 'antifungal resistance' and 'treatment of candidiasis'.

General features

The members of genus *Candida* are ubiquitous in nature and exist in both saprophytic and commensal state. [4]

They grow on variety of foods, vegetable and debris matter. *Candida* spp. are frequently isolated from skin and mucosal sites like gastrointestinal and genitourinary tract of human body.

Morphologically, they are classified as yeast like fungi whereas as per taxonomic classification, the genus *Candida* belong to the phylum Ascomycetes, class class Deuteromycetes, order Cryptococcales and family Cryptococcaceae.[4] The class Deuteromycetes is described as "taxonomic pit" in which encompass ascomycetes and basidiomycetes yeasts without known sexual stage or other remarkable phenotypic feature.[5] This genus subsumes characteristically white oval, cylindrical or elliptical asporogenous unicellular yeasts. The individual yeast cell measures about 3-5ìmx3-7ìm and is surrounded by bilayered cell-wall. *Candida* cell wall is composed of polysaccharides, mannan, glucan and chitin. The mannan protein is distributed throughout the cell wall, whereas chitin and glucan are mainly present in the inner cell wall. Vegetative reproduction occurs by budding.

Formation of true or pseudohyphae is a characteristic feature of this genus. All *Candida* spp. except *C. glabrata* and *C. parapsilosis* are capable of forming true or pseudohyphae.[4] Pseudohyphae may long, branched or elongated. *C. glabrata*, is the only *Candida* spp. that is haploid and lack the ability to produce both true and pseudohyphae whereas, *C. parapsilosis* can generate pseudohyphae but not true hyphae.[4]

The development of true hyphae is initiated by 'germ tube' formation. True hyphal formation can occur from yeast cell or even from existing hypha. True hyphae are characteristically septate.[4] The septa (cross walls) divide the hyphae into separate unit. Pseudohyphae are also originated from yeast cells or hyphae but in contrast to true hyphae they lack septa. The bud remains attach to the parent yeast cell or hypha. It further elongates and forms filaments with constriction at cell-cell junctions.[4]

C. dubliniensis is the only NAC spp. capable of producing 'germ tubes' which is the characteristic feature of *C. albicans* within 2 h of incubation in human serum or other substances like egg white, saliva, sheep serum, peptone water and trypticase soya broth.[6] This NAC spp. can also produce chlamydospores like *C. albicans*. Chlamydospores are highly refractile, round and resistant asexual spores. These are produced by *Candida* in response to unfavourable conditions.

Being a non fastidious organism, *Candida* spp. grows on most of media used for isolation pathogenic fungi. Sabouraud dextrose agar (SDA) is the most widely used for primary isolation of *Candida* spp. On this medium, *Candida* produces cream to yellow coloured colonies. The texture of colony may vary from smooth and glistening to dry, wrinkled and dull depending on species [4]. SDA is neither a selective nor a differential medium. Addition of antimicrobial agents like chloramphenicol and actidione (cycloheximide) makes this medium selective. Chloramphenicol inhibit bacterial contamination whereas, actidione prevents the growth of saprotrophic fungi [4]. However, certain strains of NAC spp. like *C. krusei* and *C. parapsilosis* are inhibited by actidione, therefore SDA with actidione is not recommended as single isolation medium.

Candida spp. can metabolize glucose either aerobically (assimilation) via hexose mono phosphate pathway or anaerobically (fermentation) via the Embden Meyerhof pathway. It ferments a number of sugars like glucose, galactose and maltose, with formation of acid & gas.

At present, there are about 200 recognized species within the genus *Candida* however, only 10% are pathogenic. Species are differentiated on the basis colony morphology, sugar fermentation and assimilation pattern. [4] *Candida* spp. implicated in human infections is shown figure 1.

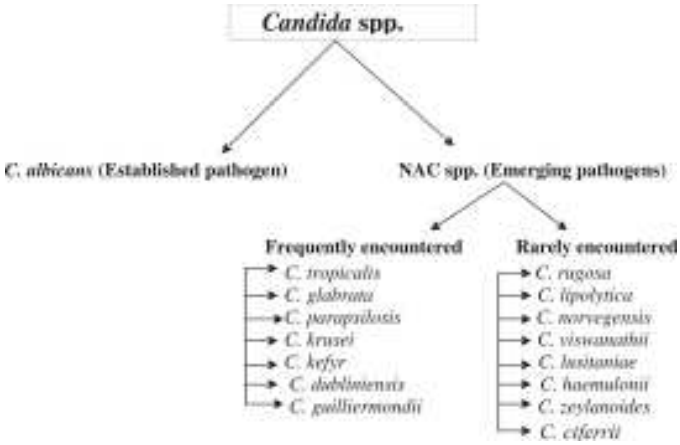

Figure1. Candida species implicated in human infections.

Epidemiology

Following the widespread use of immunosuppressive and broad spectrum antibiotic therapy, NAC spp. have emerged as an important cause of health care associated and opportunistic infections [2]. Only few decades back,

NAC spp. were overlooked as mere contaminants or non pathogenic isolates.

Candidiasis due to NAC spp. are most commonly noted in patients who on azole treatment or prophylaxis. The incidence of infections due to NAC spp. is high in haematology patients with prolonged neutropenia compared to non-neutropenic patients in the surgical intensive care unit.[7] Table 1 shows important risk factors for NAC spp. infections.

Table 1 Important risks factors for NAC spp. infections.

***Candida* spp.**	**Risk factors**
C. tropicalis	ICU stay, prolonged catheterization, total parenteral nutritional (TPN), neutropenia, malignancy.
C. glabrata	Fluconazole prophylaxis, advanced age, presence of solid tumor , bone marrow transplant recipients, abdominal surgery, renal dysfunction.
C. krusei	Flunonazole prophylaxis, leukemia, neutropenia, bone marrow transplant recipients, ICU stay, neonatal age group.
C. parapsilosis	Presence of intravascular devise, TPN, prematurity, bone marrow transplant recipients
C. lusitaniae	Bone marrow transplant recipients, cytoreductive therapy, broad spectrum antibiotics.
C. guilliermondii	Malignancy, bone marrow transplant recipients.
C. dubliniensis	HIV infection, neutropenia.

Among NAC spp., *C. tropicalis, C. krusei, C. glabrata* and *C. parapsilosis* are most commonly isolated [2]. *C. tropicalis* alone, or in association with *C. albicans* or other NAC spp. is frequently encountered in human infections. It is the 2nd or 3rd most common *Candida* spp. isolated from candidemia cases. Recent studies from India have documented *C. tropicalis* to be the most prevalent NAC spp. in *Candida* blood stream infections. *C. tropicalis* is also reported as the most common NAC spp. in candiduria patients. Mane *et al* (2010) and Kantheti *et al* (2012) reported, *C. tropicalis* as the most common NAC spp. from HIV infected patients with oropharyngeal candidiasis (OPC).[8] This NAC spp. is often isolated from ICU patients. Prolonged catheterization, antineoplastic chemotherapy, acute leukemia, neutropenia and broad spectrum antibiotic therapy are important factors that precipitate *C. tropicalis* infections.[9] As compared to *C. albicans* and other NAC spp., it has higher dissemination potential in neutropenic patients.

C. glabrata, the only known haploid species of genus *Candida* was initially considered as a saprophyte or commensal. [10] However, in recent years, this NAC spp. is increasing implicated in human infections worldwide. It is the 2nd or 3rd most common *Candida* spp. isolated from various types of candidiasis. *C. glabrata* BSI are common in older adults compared to neonatal and paediatric age group. *C. glabrata* infections are associated with high mortality rates.[9] Like any other hospital pathogen, *C. glabrata* can also be acquired, directly or indirectly, from contaminated environmental surfaces.[9]

C. parapsilosis is one of the important causes of systemic candidiasis in neonates and ICU patients. [9] This *Candida* spp. is most commonly isolated from normally sterile body sites of hospitalized patients. The ability to colonize human hands and intravascular devices and prosthetic materials, makes *C. parapsilosis* an important nosocomial pathogen.[4] *C. parapsilosis* bears selective growth ability in hyperalimentation solutions.[9] Nosocomial infection due to this NAC spp. is noted in extremely low birth weight neonates and is associated with high mortality rates.[9] It is also implicated in various other clinical manifestations like endopthalmitis, endocarditis, septic arthritis and peritonitis.[9]

C. krusei causes disseminated infections in bone marrow or stem cell transplant recipients and haematological malignancy patients.[11] The incidence of *C. krusei* BSI is high in adults, lower in children and lowest in neonates.

C. krusei is a comparatively uncommon cause of candidemia in HIV infected patients. Apart from BSI, *C. krusei* is also implicated in UTI, endopthalmitis, osteomyelitis and endocarditis. [4] Antifungal prophylaxis, particularly fluconazole is the major risk factor associated with *C. krusei* infections.[4]

C. guilliermondii is a rare cause of human infections. Initially it was considered as an animal saprophyte but in recent years, it is being isolated from BSI in patients with prior cardiovascular or gastrointestinal surgery.[12] This NAC spp. rarely causes BSI in paediatric and HIV infected patients. The majority of deaths due to *C. guilliermondii* disseminated infections occur in cancer patients.[12]

C. kefyr was reported as an emerging pathogen in patients with oncohaematological disorders by Sendid *et al* (2006).[11] Reuter *et al* (2005) reported isolation of *C. kefyr* from nosocomial BSI in neutropenic patients.[11] *C. lusitaniae* was considered as a rare pathogen before the introduction of aggressive chemotherapy in cancer patients.[12] It has emerged as an important aetiological agent in bone marrow transplant recipient and cancer patients on high dose cytoreductive therapy.[12]

C. dubliniensis, the novel *Candida* spp. was first described by Sullivan *et al* in July 1995.[13] This NAC spp. was first reported from Irish HIV infected patients with OPC. In recent years, isolation of *C. dubliniensis* is reported from other body sites/ specimens, such as vagina, urine, skin and gastrointestinal tract of both HIV infected and non infected individuals.[4] Due to limited phospholipase activity and ability of yeast-to-hyphal transformation, this NAC spp. is rarely implicated in invasive infections.[4] However, in recent years, it has been reported from few cases of candidemia.[3]

The incidence of infections due to *C. rugosa*, a relatively uncommon *Candida* spp. appears to be increased in recent years. *C. rugosa* was isolated from 1.1% of oral cavities of diabetic patients.[13] It is also implicated in catheter-related BSI in most countries.[13] *C. rugosa* is one of the common yeasts isolated from mastitic cows and hence consumption of contaminated milk or milk product may be the source of *C. rugosa* infections.

C. lipolytica, initially considered as an animal and plant saprophyte was reported as a cause of candidemia in a cancer patients who received intravenous fluconazole therapy.[12] Agarwal *et al* (2008) reported a case of catheter-related candidemia caused by *C. lipolytica*.[3]

C. pelliculosa, *C. norvegenesis* and *C. inconspicua* are the examples of NAC spp. rarely encountered from human infections. *C. inconspicua* and *C. norvegenesis* resemble *C. krusei* phenotypically.[12] Majoras *et al* reported isolation of *C. inconspicua* from respiratory tract, wound, blood and genitals.[12] Bailey *et al* (1997) reported isolation of *C. inconspicua* in a HIV infected patient.[12] Isolation of *C. norvegenesis* from clinical samples was first reported from Norway.[12] *C. norvegenesis* has been identified from respiratory specimens, blood, urine and peritoneal fluid.[12] C. pelliculosa was reported from cases of candidemia by Shivprakasha *et al* (2007).[3]

The empirical azole prophylaxis and therapy has altered the epidemiology of *Candida* infections. NAC spp. once considered as non-pathogenic and saprophytic isolates has emerged as important opportunistic pathogens. A wide variation in species distribution is noted not only in different countries of the world but also in different health care institutes within the same geographical area. Therefore institutional epidemiological factors play an important role in treatment decisions.

Clinical manifestations

Candida spp. is the only mycotic pathogen, capable of causing a wide spectrum of clinical manifestations ranging from mucocutaneous overgrowth to systemic infections. An infection caused by *Candida* spp. is referred as candidiasis. Candidiasis is usually endogenous in origin but exogenous transmission may occur in hospitals via contaminated medical devices and hands of health care providers.[4]

Candida infections are most commonly encountered in immunocompromised and critically ill, non-immunocompromised patients. Therefore, candidiasis can be rightly called as 'disease of diseased'.

Thrush, chronic atrophic stomatitis, chronic mucocutaneous candidiasis and vulvovaginitis are common manifestations of mucocutaneous candidiasis. This form of candidiasis is extremely common and occurs in otherwise healthy individuals. Mucocutaneous candidiasis is usually self limiting in healthy individuals and can be treated with basic hygiene measures. It generally responds to topical antifungal therapy. However, in case of immunosuppressed hosts, mucocutaneous *Candida* infections could become a gateway to systemic spread.[4]

The term invasive candidiasis encases two very close but distinct clinical entities: candidemia and disseminated

or systemic candidiasis. Candidemia is defined as the condition where *Candida* spp. is isolated from blood of the patient whereas disseminated or systemic candidiasis refers to a condition where *Candida* invasion is documented by positive culture or histopathological examination at non adjacent, normally sterile body site.[14]

Clinical manifestations caused by NAC spp. are usually indistinguishable from those attributed to *C. albicans*. Table 2 shows common clinical manifestations caused by NAC spp.

Table 2 Important clinical manifestations caused by NAC species.

Candida spp.	Clinical manifestations
C. tropicalis	Candidemia and other disseminated in immunocompromised patients. catheter-associated candiduria, oropharyngeal candidiasis, vulvovaginal candidiasis
C. glabrata	Candidemia, candiduria, vulvovaginal candidiasis
C. krusei	Candidemia, neonatal diarrhoea, endophthalmitis
C. parapsilosis	Candidemia, endophthalmitis, endocarditis, septic arthritis, peritonitis and other disseminated infections associated with invasive or prosthetic devices.
C. lusitaniae	Candidemia and other forms of systemic candidiasis.
C. guilliermondii	Candidemia in patients with prior cardiovascular or gastrointestinal surgery, endocarditis in intravenous drug addicts.
C. dubliniensis	Oropharyngeal candidiasis in HIV infected patients.
C. kefyr	Systemic candidiasis.
C. famata	Catheter related candidemia
C. rugosa	Candidemia and wound infections.
C. lipolytica	Intravenous catheter related candidiasis.

Pathogenicity

Until recently, the role of *Candida* spp. in the process of pathogenesis of infection was contemplated to be passive and therefore organic weakness or impairment of host's immune system were the only mechanisms pondered for establishment of this opportunistic mycotic infection. [15] However, this concept is modified and now it can be stated that, *Candida* can actively participate in the overall pathogenesis of infection using mechanisms of aggression known as virulence factors. [15]

Traits attributed for establishment and development of infection are defined as virulence factors. [4] Different virulence factors collectively attribute to the microbial pathogenicity. Various virulence factors like adhesion to host tissues, biofilm formation and secretion of extracellular hydrolases contribute to the pathogenicity of *Candida* spp. [4] Most of published literatures on virulence factors of *Candida* spp. are focused on *C. albicans* and hence relatively less is known about NAC spp.

Adherence to host cell is the first and most important step in the establishment of infection. Adhesion aid *Candida* to penetrate and disseminate the host tissues. *Candida* can adhere to variety of cells including vaginal, gastrointestinal and buccal epithelium cells, endothelium, polymorphs and lymphocytes. [4] In *Candida* spp. adhesins (specialized surface proteins responsible for adhesion) can recognize host ligands (binding site) like proteins, fibrinogen and fibrinonectin. Several studies have documented high adhesive property in *C. albicans*.

In *C. albicans*, a major group of adhesins are coded by the *ALS* (agglutinin like sequence). In recent years, few researchers have directed their studies towards adhesive properties of NAC spp. Among NAC spp., *in vitro* adherence is demonstrated in *C. tropicalis*, *C. glabrata* and *C. dubliniensis*. Gilfillan *et al*, McCullough *et al* and Jabra-Risk *et al* reported *C. dubliniensis* to be more adherent than *C. albicans*. [16] Adhesins of *C. dubliniensis* are encoded by the gene similar to *C. albicans*. However, the regulation of genes may be different in these two species. [16] Adhesins of *C. glabrata* is encoded by the *EPA* (epithelial adhesin) gene family. [9] Epa proteins are structurally similar to Als proteins of *C. albicans*. Number of genes responsible for adhesion in *C. parapsilosis* has been identified. [9] These include five Als proteins and six Pga (predicted glycosyl phosphatidyl-anchored protein 30). At least three Als protein have identified in *C. tropicalis*. [16]

Ability to form biofilms on medical devices is an important virulence factor responsible for emergence of *Candida* spp. as one of the important causes of health care associated infections (HCAIs). Biofilms are surface-associated microbial communities which are firmly fixed within an extracellular matrix. [15] It limits the penetration of an antifungal agent through the matrix and protects the yeast cells from host immune response. The ability to form biofilm and extracellular matrix varies according to species, strain and environmental factors. Biofilm matrix of *C. albicans* is made up of carbohydrates, proteins, phosphorus and hexosamines. *C. tropicalis* biofilm matrix contains low levels of carbohydrates and proteins compared to *C. albicans* and other NAC spp. [9] *C. glabrata* biofilm matrix has high levels of proteins and carbohydrates. [4, 9] The extracellular matrix of *C. parapsilosis* biofilm is mainly composed of carbohydrates and low levels of proteins. [4, 9] Biofilm formation is noted in NAC spp. like *C. tropicalis*, *C. glabrata*, *C. parapsilosis* and *C. dubliniensis*. Deorukhkar *et al* (2014) noted greater biofilm forming ability in *C. tropicalis* compared to *C. albicans*. [8]

Extracellular hydrolytic enzymes play a crucial role in pathogenesis of candidiasis. In *Candida* spp., the invasion of host tissue is facilitated by enzymatic activation of phospholipases, lipases and proteinases. Enzyme phospholipase hydrolyses phospholipids of host cell membrane and expose receptors to facilitate the adhesion of yeast cells. [4] Several studies have reported phospholipase activity in NAC spp. like *C. tropicalis* and *C. parapsilosis*. In contrary to *C. tropicalis* and *C. parapsilosis*, very few studies are available on phospholipase production in *C. glabrata*. [4, 9] *C. dubliniensis* isolates often demonstrate low phospholipase activity. This could be one of the possible reason for minimal or no role of this NAC spp. in invasive infections. [2]

In addition to phospholipases, secreted aspartyl proteinases (saps) play a pivotal role in adherence, penetration, dissemination and the destruction of host tissues. These enzymes degrade important immunological and structural defence proteins of host. Proteinase activity is described in NAC spp. like *C. tropicalis*, *C. parapsilosis* and *C. guilliermondii*. [4, 9] *C. dubliniensis* isolates demonstrates high proteinase activity compared to *C. albicans*. [16]

Haemolysin is an example of protein that plays an important role in virulence of *Candida* spp. This protein destructs host erythrocytes and facilitates hyphal invasion. Haemolysin is essential for survival and persistence of *Candida* spp. in the host. [4, 8] Most of NAC spp. are able to produce haemolysins *in vitro*, although the extent of haemolytic activity is both strain and species dependent. [8]

NAC spp. can produce virulence factors once only attributed to *C. albicans*. The type of virulence factors produced varies with species, strain and site of lesions and therefore identification of these virulence attributes unique to a particular *Candida* spp. is absolutely necessary to understand the pathogenesis and epidemiology of *Candida* infections. In near future, the virulence factors may provide powerful insights for development of new antifungal drug targets.

Antifungal Resistance

Antifungal resistance once rarely reported in *Candida* spp. has emerged as important health care problem worldwide. Various factors like the advent of HIV/AIDS and the widespread use of azole group of antifungal agents to treat and prevent mycotic infections in these patients have significantly contributed to emergence of antifungal resistance in *Candida* spp. Empirical use of azoles has increased the incidence of infections due to relatively unknown, unusual and treatment resistance *Candida* spp. [14] The classification of antifungal resistance is shown in figure 2.

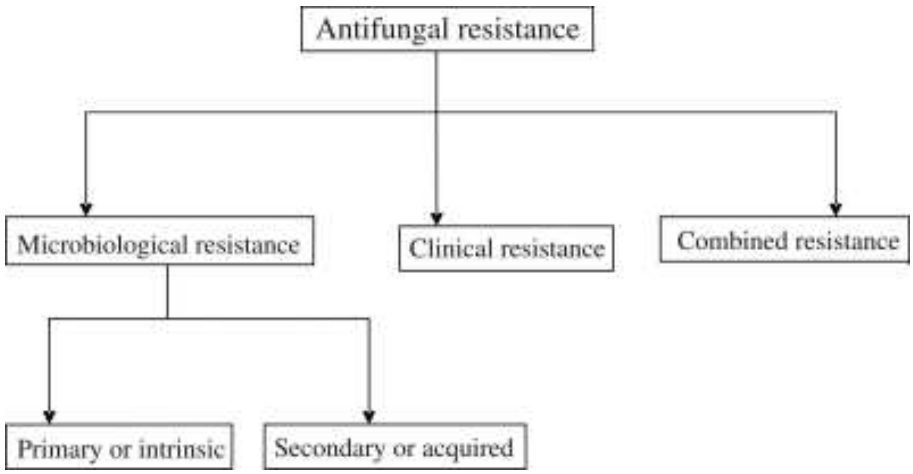

Figure 2. Classification of antifungal resistance.

Microbiological or microbial resistance refers to the condition where *in vitro* susceptibility testing demonstrates non susceptibility of a fungus to an antifungal drug. It may be also defined as the condition in which the minimum inhibitory concentration (MIC) of the antifungal drug exceeds the susceptibility breakpoint for that fungus. [17] Microbial resistance is further classified into primary or intrinsic resistance and secondary or acquired resistance. [4]

Primary (intrinsic) resistance is noted naturally or innately among certain fungal pathogens without prior exposure to an antifungal drug. E.g. fluconazole resistance in *C. krusei*. [4] Secondary (acquired) resistance refers to resistance acquired by the previously susceptible fungal strain after exposure to an antifungal drug. This type of resistance is usually dependent on altered gene expression. [4] E. g. development of fluconazole resistance in *C. tropicalis* and *C. dubliniensis* isolates during course of treatment. [4]

As shown in Figue 3. development of resistance to a particular antifungal agent by a fungal pathogen is dependent on multiple factors like properties of infecting fungal pathogens, pharmacokinetic and pharmacodynamic properties of antifungal drugs and host's predisposing factors.

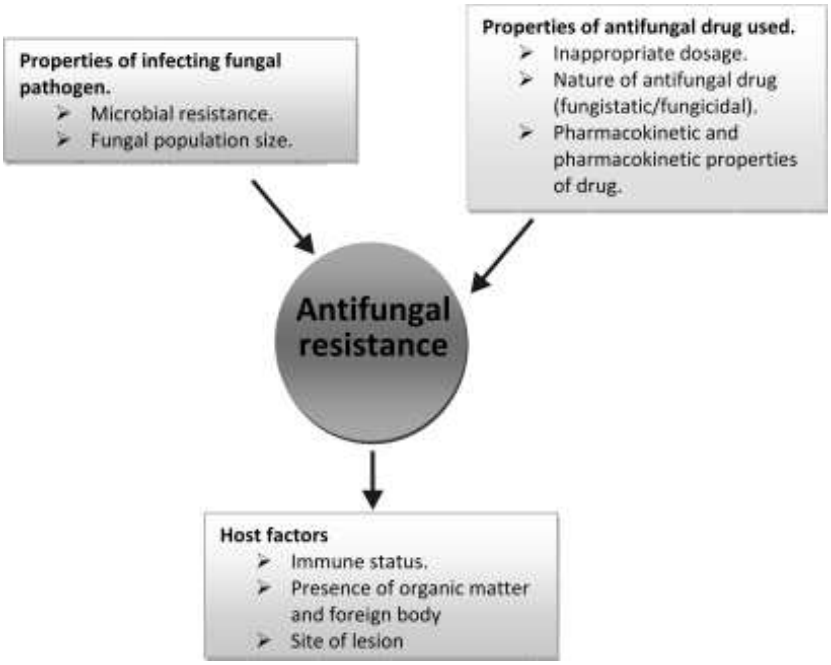

Figure 3. Factors contributing to antifungal resistance.

The number of available antifungal drugs is limited compared to antibacterial agents. Antifungal agents are classified into polyenes, azoles, pyrimidine analogue and echinocandins. [18] These drugs differ in their mode of action, pharmacokinetic and pharmacodynamic properties and route of administration. [4] Table 3 shows properties of commonly used antifungal drugs for prophylaxis and treatment of candidiasis.

Antifungal agent	Nature	Mode of action	Route of administration
Polyene Amphotericin BNystatin	Fungicidal	Distruption of fungal cell wall	IV Topical
Azoles Fluconazole Itraconazole Voriconazole Posaconazole	Fungistatic	Inhibition of ergosterol biosyntheisis by blocking lanosterol 14á-demethylase.	IV and oral IV and oral IV and oralOral
Echinocandins Caspofungin Micafungin Anidulafungin	Fungistatic or fungicidal	Inhibition of â-(1-3)-D-glucan by binding to â-(1-3)-D-glucan synthase.	IV IV IV

*IV-intravenous

Amphotericin B, the broad spectrum polyene antifungal is used for treatment of disseminated *Candida* infections. [7] This antifungal drug has unique mechanism of action. Amphotericin B unlike many other antimicrobial agents binds to ergosterol, the principal sterol in the fungal cell membrane and causes leakage of cytoplasmic contents. [4] Resistance to amphotericin B is very rare. *C. albicans* and most of NAC spp. are considered to be amphotericin B susceptible. However amphotericin B resistance is occasionally reported from few strains of NAC spp. like *C. tropicalis, C. krusei* and *C. lusitaniae*. [4] *C. glabrata* is considered as intermediate or susceptible dose dependent. [19] Mechanisms like decrease in ergosterol content in the plasmalemma or alteration in the target lipid leading to a decrease in the amphotericin B binding have been proposed for amphotericin B. [19] In *Candida* spp. polyene resistance is usually a result of defective ergosterol biosynthesis.[19] Mutation in genes like *ERG3, ERG11* and *ERG6* leads to polyene resistance. [19] Amphotericin B resistant NAC spp. are associated with a poorer outcome than susceptible strains.

Azole antifungal drugs (especially fluconazole) are more frequently used prophylaxis and treatment of *Candida* infections. This group of antifungals inhibits C-14 á-sterol demethylation (the primary step in ergosterol synthesis) leading to accumulation of methylated sterols and finally the cell membrane disruption. [19] Widespread empirical use of this group of antifungal agents is thought to be the major factor for emergence of azole resistance and azole resistant NAC spp. Decreased drug concentration, alteration in target site, upregulation of target enzymes and development of bypass pathways are major mechanisms described for azole resistance in *Candida* spp. [4] *C. krusei*, an emerging pathogen from NAC spp. is intrinsically resistant to fluconazole. [4] Few *C. glabrata* isolates are intermediately resistant to azole antifungals whereas 20% of strains develop secondary resistance during fluconazole treatment or prophylaxis. [12] *C. tropicalis* was initially considered as fluconazole susceptible species. However, recent studies have reported development of fluconazole resistance in *C. tropicalis* isolates. *C. dubliniensis* although the innately fluconazole sensitive species can rapidly acquire resistance during course of therapy. [2]

The echinocandins (caspofungin, anidulafungin and micafungin) are recent addition to antifungal arsenal. This group of antifungals inhibits the synthesis of â-1-3-D-glucan, the important structural and functional part of fungal cell wall. [7] Mechanisms like insufficient production of target enzyme â-1-3-D-glucan synthase or generation of alternate form of the enzyme with

reduced drug binding are suggested for echinocandin resistance in *Candida* spp. [20] Echinocandins demonstrate fungicidal activity (both *in vivo* and *in vitro*) against *C. albicans* and commonly isolated NAC spp. like *C. glabrata* and *C. tropicalis*.[4] However *C. parapsilosis* isolates have higher minimum inhibitory concentration (MIC) for echinocandins compared to *C. albicans* and other NAC spp.[4]

Conclusion

Invasive infections due to Non-albicans *Candida* have emerged as important cause of morbidity and mortality among hospitalized patients. As compared to *C. albicans*, these emerging NAC spp. isolates demonstrate considerable reduced susceptibility to commonly used antifungal drugs. The *Candida* isolates belonging to NAC spp. differ in the terms of epidemiology, pathophysiology and of most important the pattern of susceptibility to a particular antifungal drug. Therefore species identification once seldom performed in clinical microbiological services for *Candida* spp. has became important for 'species directed therapy'. The *in-vitro* susceptibility testing often guides clinicians in selection of appropriate antifungal therapeutic agent.

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