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Gene Responsible for Emergence of Antifungal Resistance in Candida Spps. & Diagnosis: A Review

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Abstract

Candida is a commensal & present in moist region of the human body. It also acts as an opportunistic pathogen that is prevalent in immunocompromised patients such as in case of HIV, hepatitis B, Cancer or also in diabetic patients. In COVID19 era, it also causes no. of cases in COVID19 patients due immunosuppression. It also known by name i.e. white fungus. The numbers of cases mainly increase in patients who are hospitalized due to the COVID19, because in hospital there are number of resistance organisms which are present in hospital environment.C. auris is reported in India but also in all over the world. Rise of antifungal resistance in Candida also become a big problem for public health sector. Due to this, Candida acquired resistance against azole, polynes & echinocandins. This resistance mechanism increased due to over expression of genes such as SNQ2, TPO3, ABC1, ERG11, ERG2, ERG3, ERG5, ERG6 which gain in function through mutation in the transcription that leads to alteration in gene expression that cause increase in drug efflux pumps, increased concentration of lanosterol 14α-demethylase & point alteration in which ergosterol content decreased in cells. Diagnosis can be done by multiplex PCR technique, to know the gene which responsible for antifungal drugresistance in that particular Candida specie.

Keywords: Candidemia; Antifungal drugs; COVID19; Co-infection; CHROMagar.

Introduction

Fungal infection are commonly neglated in the presence of bacterias& viruses but there are 1.5 million fungal species in which 300 causes human diseases & 300 million people are infected by these invasive fungal pathogens & 1.6 million die annually due to these pathogens including Aspergillus, Norcardia, Mucormycosis, cryptococcus, Bascidomycosis, Pneumocystis, Candida & many more.[1] Candida is a Dimorphic Fungi & also called as Yeast like fungi, which present as a commensal in human body at moist region i.e. gastrointestinal, genitourinary tracts and in the oral and conjunctival flora.[2]

Candida genera contain mainly 200 species in which the medically important species includes *Candida albicans, Candida glabrata, Candida tropicalis, Candida parapsilosis, and Candida krusei* & all these are responsible for 90% invasive infections in humans but there are some species which also emerge as pathogesn such as *Candida guilliermondii, Candida kefyr, Candida rugosa, Candida dubliniensis, and Candida famata.*

It have three main cellular morphologies:-yeast, pseudohyphae, and hyphae. Yeast are single cells that are oval in shape and divided by budding when grown at room temperature. Pseudohyphae and Hyphae are filamentous form which grown in polarized manner in host body or at 37°C. This form

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also provide it another virulence factor because in hyphal form are able to release hydrolytic enzyme such as phospholipid & proteinase which help to invade the organism to adjacent tissue specifically in endothelial & epithelial cells.[3,7]

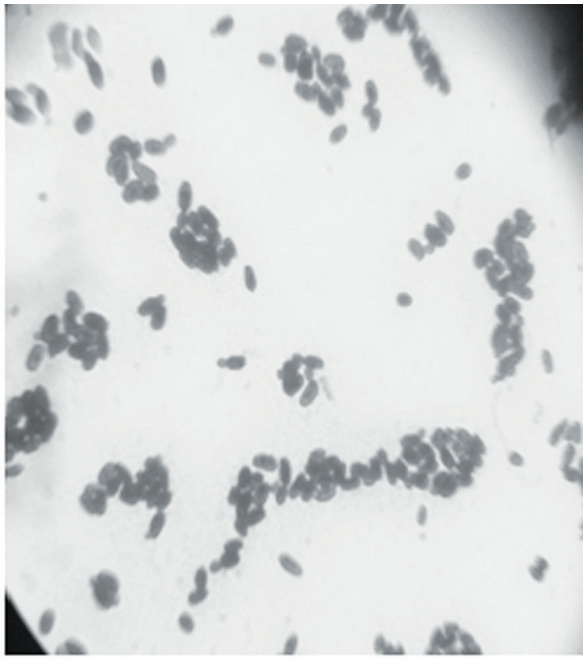

Fig. 1: microscopic view of Candida under light microscope.

Candida a commensal not allows the other organisms to settle down in body which act as defense for invading organisms but in an immunosuppressive conditions such as in cases of HIV, Hepatitis, Cancer, Diabetic condition, Chemotherapy, Organtransplantation & even in era of COVID19, it act as an invasive organism which causes co-infection in patients mostly to those who area dmittedtohospitals.

Candida causes 3 type of infection such as superficial, Subcutaneous & invasive infection with a mortality rate of 45%, according to Nosocomial Infections Surveillance System (NNISS) reported that Candida cause four the number of blood Infection known as Candidemia which is life threatening Infection.[4]

Superficial Candidasis are most common in several countries but, its cases are high in tropical & sub tropical countries. This includes commonly infections of mucosal layer of human body such as nail, vagina, oralthrush, paronychia & inter digital candidiasis on hand or foot.[5]

Subcutaneous candidiasis infection of humans results from inoculation of Candida in tissues which results from trauma, injury or hematogenous spread & these infections also responsible for invasive infection.[6]

Invasive candidiasis infection increased significantly in Immune compromised population who are hospitalized due to Nosocomial Infection.

Table 1: Most prevalent type of infections of Candida in humans.

Candida Infection Rate

Rank	Type of Infection
3 -4th	Most isolated nosocomial blood stream infection.
4th	Most common Hospital Acquired Systemic Infection.
5th	Most common cause of blood stream infections in pediatric intensive care units.

It's risk factors includes hematological malignances, bone marrow transplantation, prolong treatment with corticosteroid, in Intensive care, chemotherapy, HIV infection or incase of malnutrition & in severe burn.[7]

Invasive candidias is an important health issue that caused by several Candidaspps. Commonly *C.albicans* i.e. 70 percent but varies according to geographical condition. Through bloodstream it spread to other organs such as the liver, heart, kidney, spleen & brain.[8]

Mainly the infection of Candida to human are prevalent in hospitalized people who already surffed from another disease & in present scenario it also seen in many patients. When human immune suppressed due to this disease then self & environment organism try to override the human body that leads to Co-infection & with this hospital environment may also contains antibiotics Resistance organisms which cause disease in human. And anti fungal resistance Candida also responsible for infection in this kind of situation, through Horizontal Gene Transfer it also transfer gene which spread resistance to other organisms & make the patient a Carrier who can also responsible for the spread of community acquire infection when it was discharged from hospital.

There are many antifungals used to treat Candida infection & mainly are azoles antifungals which are easily available, inexpensive & exhibits limited toxicity but due to over & unwanted use of these drugs increased the emergence of antifungal resistance Candida. Azoles resistance infection caused by *Candida albicans* and but this resistance also emerge in non albicansspps.[9]

There are also several classes of compounds used for treatment of Candida infections which explains in below table.[10-17]

Table 2: Various classes of antifungal with their different properties.

Classes	Drugs	Dosage		Mechanism of effect	Mechanism of resistance	Toxicity
		IV	Oral			
Azoles	Fluconazole	400–800 mg/d 100–200 mg/dc	400–800 mg/d 100–200 mg/dc	impair ergo sterol synthesis by inhibiting C14-a sterol demethylase that lead to disruption of sterol precursors & reduction of registered in cell membrane.	a) Alteration in ERG 11 & THR 1 gene cause modification in quality & quantity of 14a-demethylase in the expression of resistance to azole antifungal agents. b)the up regulation of efflux pumps, has also promote drug resistance via a decrease in intracellular drug levels.	the most common side-effects include rash, headache, or gastrointestinal upset &Hepatotoxicity.
	Itraconazole	NA	200 mg 1-3/d	-	-	-
	Voriconazole	6 mg/kg for 2 doses, then 4 mg/kg q 12 h	400 mg bid for 2 doses, then 200 mg q 12 h	-	-	-
Polynes	Ampho-tericin B	0.7–1 mg/kg/ da	NA	Interaction with membrane sterols results in production of pore that leads to altered permeability & leakage cause death of organisms.	Defects in the ERG3 gene involved in erogosterol biosynthesis lead to accumulation of other sterols in the fungal membrance.	Adverse effects include renal toxicity, infusion reactions, electrolyte abnormalities, and hepatotoxicity.
Allylamines	Naftifine	NA	250 mg day or topical (1% cream) administration	inhibit ergo sterol synthesis at the level of squaleneepoxidase with highly selective for the fungal enzyme but minimal effect on mammalian cholesterol synthesis.	Not yet reported	Patients experiencing AE, including mild burning/stinging, itching, erythema, irritation, and rarely, allergic reactions.
	Terbinafine	NA	7–12.5 mg/kg/ day	-	-	-
Echino-candins	Caspofungin	70 mg for 1 dose, then 50 mg/d	NA	compounds disrupt the fungal cell wall by inhibiting the synthesis of b-1,3 glucan which a fungal cell polysaccharide.	Hot-spot mutations in FKS1 or FKS2 genes cause change in amino acids substitution of fks subunits of glucansynthase.	adverse reactions include gastrointestinal upset, headache, elevation of liver (aminotransferase) tests, or mild infusion reaction.
	Micafungin	100–150 mg/d 50 mg/d	NA	-	-	-
	Anidulafungin	100–200 mg for 1 dose, then 50–200 mg/d	NA	-	-	-

Antifungal activity can be measure with standard dilution in liquid media or with solid surface agar with drug gradient can be used. Two major protocols are currently used i.e CLSI (clinical laboratory standard institute) & EUCAST (European committee on antimicrobial susceptibility testing), there protocols yields called MIC (Minimum inhibitor concentration).[18] MIC is the lowest concentration (mg/l) of antibiotics which is able to inhibit the growth of organism & also helpful to know the resistance or susceptibility of organism for against a one or more antibiotics.

MIC play important role in new antifungal drugs which used in vitro testing that can predict the effects of compounds in vivo & further clinical results used for treatment.[19]

Mechanisms leading to the emergence of

resistance includes single-point mutations chromosomal rearrangements & horizontal gene transfer (HGT) or hybridization but in all these HGT is most responsible for transfer of gene which are associated with resistance emergence.[20]

Table 3:. Different type of gene found in different - different candida species which are responsible for emergence of antifungal resistance.

Antifungal Classes	Species	Gene
Azoles	*C. albicans*	ERG11
		UPC2
		TAC1
		MRR1
		ERG3
		CDR1
		CDR2
		SNQ2
		ABC1
		MDR1
		TPO3
	C.glabrata	MDR1
		TPO3
	C.parapsilosis	ERG11
		CDR1
		CDR2
		SNQ2
		ABC1
		MDR1
		TOP3
	C.tropicalis	ERG11
		CDR1
		CDR2
		SNQ2
		ABC1
		MDR1
		TPO3
	C.kruse	ERG11
		CDR1
		CDR2
		SNQ2
		ABC1
		MDR1
		TPO3
	C. auris	ERG11
Echino-candins	*C. albicans*	FKS1
		FKS2
Polyenes	*C. albicans*	ERG2
		ERG3
		ERG5
		ERG11
	C. glabrata	ERG2
		ERG6

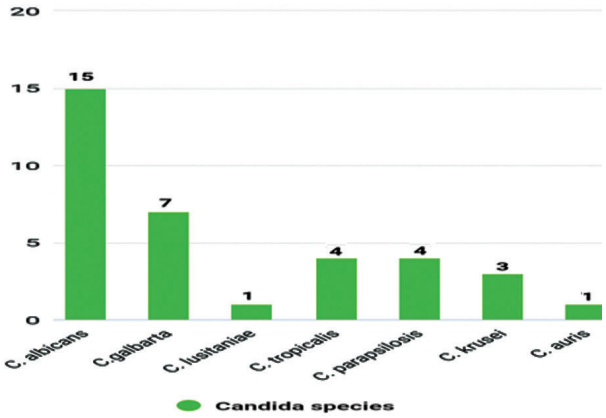

Fig. 2: Graphical representation of no. of gene present in different Candida species.

Co-infection of Candida is seen in COVID 19 patients since the pandemic started & co-infection also named as super infection. *C. glabrata* a common fungal commensal of mucosal surface that cause blood stream infection in some countries includes USA, Asia & European countries with mutidrug resistance that shows the high tolerance of Candida spps. Against different classes of antifungal drugs.[21] SARS-CoV2 is responsible for pandemic all over the world which effect approximately 196 countries people & started as epidemic in Wuhan, China. Wuhan virus isolated from epithelial cells of nasopharynx from a cluster of patient with pneumonia like symptoms. SARS-CoV2 belong to corona virus family but it is different from both MERS-CoV& SARS-CoV, these also infect humans. It emerges as global pathogen which challenges the whole world public health sector. Before SARS-CoV2 pandemic start, SARS-CoV also outbreaks in 2002 & 2003 in changing province china while MERS-CoV was outbreaks in 2012 in middle East. In late Dec 2019, SARS-CoV2 outbreaks all over the world which lead to biggest pandemic of the century after the Spanish flu 1920 which responsible for death of 50 million people in all over the world.[22]

During April-July 2020, 15 patients who was critically ill due to COVID19 they are affected with candidemia which was caused by C.auris with 60 percent high case fatality rate.[23] COVID19 patients are prone to respiratory disease syndrome which are acquired by patients through ventilators in ICUs & this also reported in 40 countries across 6 continents that's why it also known as environmental colonizer of ICUs but diagnostics resources are limited in another countries.[24]

Primary antifungals are failed against the multidrug resistance candidaspps. Such as *C.auris & C.glabrata* which increase the demand for

development of new antifungals with different novel action mechanism against organisms.[25]

Diagnosis of Candida spps. infection done by conventional detection method that based on blood & culture plate method which is time consuming and take 2-4 days to identify Candida spps. While nonconventional method include serological & nucleic acid detective test. For diagnosis specimen collected from site of infection such as nail clippings, skin scrapings, tissues, aspirates and respiratory specimens, microscopic observation and detection can be used, whereas for fluidic samples such as blood and urine.[26] For isolation of Candida, specimen is cultured general in Sabouraud dextrose agar (SDA) & Potato dextrose agar (PDA) but for advance diagnosis today CHROMagar is nobel media for isolation & differentiation of Medically important Candida species.[27]

CHROMagar allow selective identification of Candida and on the basis of color reactions and colony morphology.

- *C. albicans* gave distinctive apple green color colonies.
- *C. galbrata* gave dark pink colonies with pale edges.
- *C. tropicalis* gave metallic blue colonies.
- *C. krusei* gave lavender color colonies but had velvety texture.

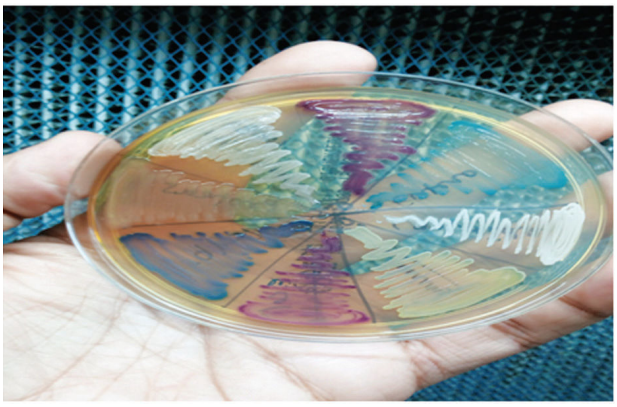

Fig. 3: Different type of organism give different color colonies in CHROMagar.

Each species also gave rise to a variety of colonies colors ranging from pink to green to blue of different colony characteristics later identity of all the isolates was confirmed with biochemical tests.[28,29]

Swab inoculated in SDA or CHROMagar but in case of tissue, they were stained with calcuoflouer white stain (CFW) & Acridine orange (AO) stain. CFW is non specific fluorochrome stain that binds to fungi while AO is metachromatin stain that selective for nucleic stain.[30]

Germ-tube test used in the rapid identification that revealed in 2-hr with 87.1% sensitive and 100% specific for the identification of C. albicans.[31,32]

Now days Polymerize chain reaction which is most rapid test for detection of any Candida spps. On the basis of gene difference & also able to differentiate the spps.. For C. albicans, single pair of primer SC1F & SC1R use that amplifies a 670 bp fragment of KER1 gene.[33] After isolation of genomic DNAs from Candida spps., species specific primers used for repetitive sequence for PCR amplification.[34]

On the basis of sequence data from ITS1 & ITS2 regions of reference strain from Candida genus which are available in EMBL/GeneBank databases, the species-specific primers, Calb, Cgla, Ckru, Cpar, Ctro, Clus, Cgni & Club were designed for specifically identify 8 clinical associated candidaspps. *C.albicans, C.Krusei, C. galbrata, C. parapsilosis, C. tropicalis, C. guilliermondii, C. lusitaniae and C. dubliniensis* respectively. These are yeast specific universal primers UNI1 & UNI2 used to amplify regions 1 (ITS1) & (ITS2) that are mostly associated with disease causing Candida.[35]

Result

ERG11 is prevalent in all of genes which are responsible for emergence of antifungal resistance in Candida & spread through the mode of horizontal gene transfer with in case of hospital admitted patients that may also lead to community acquired infection because after discharge patients act as carriers in society.

Conclusion

All the classes of antifungal are now resistance, due to the emergence of large no. of genes such as ABC1,SNQ1,TPO3,ERG3, ERG5, ERG6 or many more which provided the resistances to Candida in few decades that become big problem all around the world health sector. Ignorance of fungus infection with over use is also responsible for this anti fungal resistance because there have not action plane against the emerging resistance. There is lots of necessity of new age antifungal that help to counter the antifungal infection with new mode of action of drugs which plays important role. Early diagnosis also plays important role because with proper & correct antifungal drugs treatment can decreased the chance for development of antifungal cases. For this CHROMagar & multiplex PCR plays

important role in advanced diagnosis of early Candida infection.

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