

REVIEW ARTICLE

CANDIDIASIS IN PERSPECTIVE OF PAKISTAN

Rabea Rizwan¹, Zahida Memon², Shehla Shaheen³

¹Department of Pharmacology, Ziauddin University, Karachi.

ABSTRACT

Candidiasis is one of the common fungal infections. It is known as the disease of people with compromised immunity, that usually infects premature infants, elderly and hospitalized patients with co-morbidities. Although a concise national data is unavailable on prevalence of candidiasis in Pakistan but few studies conducted in tertiary set ups have shown prevalence of albicans around 56-59%. WHO has labelled Pakistan having the highest global burden of candidemia (invasive candidiasis) which is exceedingly fatal.

Few hospital-based studies displayed vaginitis as frequently occurring candidiasis, ranging from 18 to 38%. While the resistance against commonly used antifungal drugs varies from 34 to 61%. It is noticed that due to environmental variations fungal infections are increasing day by day but at national level no surveillance program has been developed so far.

The current review was undertaken to highlight the issues related to incidence, causes, culture and sensitivity pattern of candidiasis in Pakistan and to identify the gaps in this domain. There is intense urge to formulate new drugs or alternative agents with better efficacy, safety and affordability to combat this slowly growing enemy (candida). Therefore, preventive steps should be taken to decrease its spread, especially in hospital settings which are the main harbor of these infections.

KEYWORDS: Fungi, Candidiasis, *C. albicans*, fluconazole

Corresponding Author

Rabea Rizwan

Department of Pharmacology,

Ziauddin University

Email: rabea_rizwan@hotmail.com

INTRODUCTION

Fungi are the eukaryotic organisms. They were discovered 1,600,000 years back. These organisms contain nucleus like mammalian cells but the main difference is that their cell wall constitute of chitin. Fungal organisms exist in broad diverse form round the globe. Approximately 1,500,000 it's species have discovered out of which 300 species are considered as pathogens for human beings ⁽¹⁾.

The occurrence of fungal infections have been significantly increasing since last ten years ⁽²⁾. They are considered as one of the greatest challenging infectious agents to treat humans. The three-principal human infecting fungal phyla are;

- Entomophthoromycota (e.g. *Basidiobolus* and *Conidiobolus*)
- Ascomycota (e.g. *Candida*, *Aspergillus fumigatus*),
- Basidiomycota (e.g. *Cryptococci*) ⁽³⁾.

Other than phyla, pathologically they are also classified as primary and opportunistic organisms, in

the form of mold, filament or yeast for example *Candida* and *Aspergillus* ⁽²⁾. Every year, globally, 1,500,000 people acquire superficial and deep invasive fungal infections, which has claimed 90 to 95% lives of the people who are infected, as shown in table 1 below.

Table 1. Global incidence and mortality rates of fungal infections per annum (4).

Common fungal infections	Predicted reports every /year	Predicted death rates / year(%)
Candidiasis	> 1 million	10 to 75
Cryptococcosis	> 4 lacs	20 to 70
Aspergillosis	> 2 lacs	30 to 95

Candidiasis is highly prevalent all over the world and associated with significant morbidity and mortality as shown in table 1. As evident from individual hospital records, Pakistan has higher prevalence of candidiasis and associated complications, still no concise national data is available to elaborate the magnitude of problem exactly.

OBJECTIVES

The current review was undertaken to emphasize the issues related to rising wave of candidiasis, its causes, and culture and sensitivity patterns in Pakistan. This will help to identify the current lacunae and will also guide to chalk out the solutions in this highly neglected area of research in our country. For this purpose, the global and national data related to fungal infections was gathered from the scholarly articles published in peer reviewed journals from last 7 to 10 years (from 2010-2017) and were retrieved from Google Scholar, Pub Med, PakMediNet and journals' websites.

DISCUSSION

PREDISPOSING FACTORS OF FUNGAL INFECTIONS:

The global warming tapers the temperature difference i-e, gradient between the environmental and human body temperature (our normal body temperature above normal room temperature protects us from pathogens infecting us) and hence fungi are becoming more temperature resistant with time ⁽¹⁾. Therefore, a day by day increased global warming has led to increase in the risk of fungal infections.

As it is known, that, Pakistan is present in the north temperate (subtropical) zone of this planet having seasonal variations in different areas. Due to the global warming impact, Pakistan is greatly affected by the sudden climate changes. It is one of the humid country with summer and variant winter monsoon seasons. Annually, Pakistan has been facing the problem of floods, overfilling of rivers and dams, secondary to usual heavy rainfalls and melting of glaciers (from Himalaya and Karakorum ranges) ⁽⁵⁾. Presence of all these factors have made Pakistan, a highly vulnerable geographical location for fungal infections.

According to various literatures' surveys besides the

global warming, the incidence of other risk factors and causes of fungal infections are also increasing with time ⁽⁶⁻⁸⁾. Modification of human life style, continuous rise in medical advancements, development of medical facilities and invents have tremendously improved the survival of severely and chronically ill patients which subsequently have expanded the population of immunocompromised people e.g. organ transplant, rheumatoid and leukemia patients. All of these people, along with the use of antimicrobials, immunosuppressants, chemotherapeutics, radiation therapy and systemic as well as local steroid therapy are at higher risk of acquiring candidiasis ⁽⁹⁾. According to a report of WHO regarding candidiasis in Pakistan, people with co-morbidities such as diabetes mellitus, tuberculosis, cancer or suffering from chronic respiratory illness have greater risk of getting candidal infections ⁽¹⁰⁾.

CANDIDA AND ITS SPECIES:

Among the yeast infections affecting human beings, the most common is Candidiasis. It acts as a dimorphic organism resident as normal flora on our skin epithelial and mucosal surfaces, under favorable situations, especially due to host's altered immunity, the organism takes the advantage and can invade inside the body by forming pseudohyphae and hyphae. It can infect from superficial mucocutaneous tissues as well as can proliferate inside and infect the blood, resulting in systemic candidemia. Furthermore, it can invade through the blood to the organs that could become highly fatal ⁽¹¹⁾.

Till date *C. albicans* is the most prevalent species, responsible for candidiasis out of its 200 discovered species. Among systemic candidiasis it is responsible to cause around 50 -70% of candidal infections. However, in the recent times incidence of species is changing and the incidence of non-candida albicans (NCACS) is rising progressively. Other common species of candida infecting humans are shown below in figure 1 and 2. ^(12, 13)

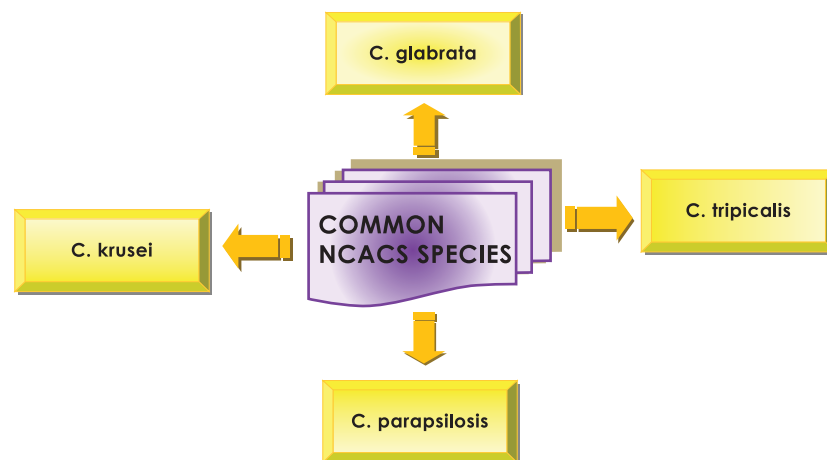


Figure 1: Common infecting Candidal species other than candida albicans (*C. albicans*). ^(12, 13)

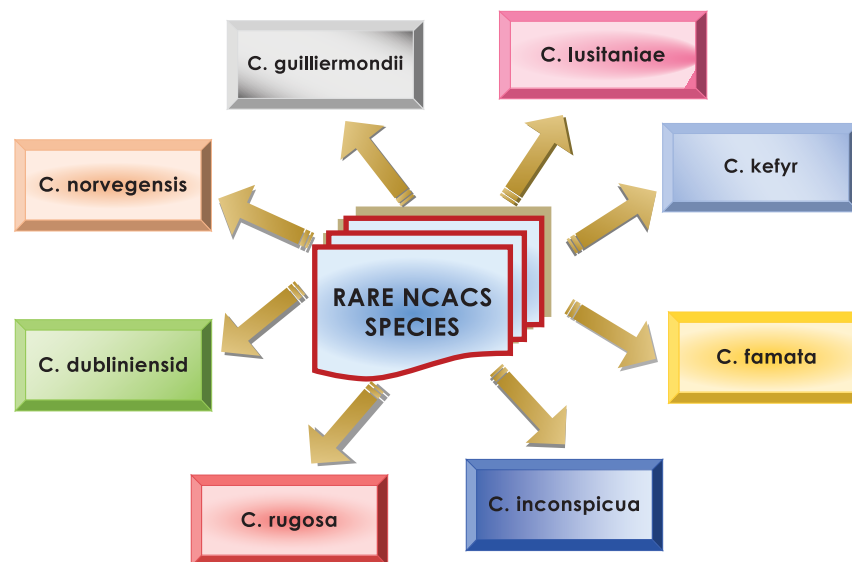


Figure 2: Rare infecting non candidal species (NCACs) to humans. ^(12, 13)

GLOBAL PREVALENCE OF CANDIDIASIS:

Worldwide diverse geographical and environmental variations have been observed in the prevalence of candidal species. *C. albicans* have been described as predominant species in United States and Europe (north and south of the continent), whilst non *albicans* in other rest of the continents, that are;

- C. glabrata* in center of Europe,
- C. parapsilosis* in Asia, south of America and Europe
- C. tropicalis* in east of Asia and Argentina ⁽¹²⁾.

CANDIDIASIS AND ITS PREVALENCE IN PAKISTAN:

According to census 2013 and 2016, Pakistan is the sixth densely populated state round the globe (185 to 203.4 million) ^(14, 15). There is a high load of transmissible and non-transmissible illness in Pakistan and fungal diseases are also one of them, but proper documentation is lacking to state burden of fungal disease exactly. These organisms causing systemic invasive infections are currently recognized as 'hidden killers' due to the high mortality rates associated with these infections which are considered and assessed as parallel to tuberculosis and malaria ^(10, 16).

Few and limited studies conducted in tertiary care settings in the country on the candidal yeast showed the most frequent risk factors responsible are hospital acquired infections and weakened immune patients with prolonged catheterization and on polypharmacy ^(17, 18).

Two studies conducted in hospital settings of Rawalpindi and Islamabad during the year around 2014 – 2016, displayed that majority of candidiasis obtained, were isolated from urine (35.6% - 38.6%),

followed by vulvovaginal (18.3%- 26.9%), salivary swabs and sputum (35.6%), other common sites of these isolates were from the sites of indwelled catheters and body fluids aspirates. Most of the cases of candida were reported in these hospital settings were general medicine ward (52%) followed by intensive care units (18%). In both of the studies, candidiasis was found more common in female gender from 34.2% - 56% while the most common species isolated was *C. albicans* (55.5% to 58.45%) ^(13, 18).

INVASIVE CANDIDIASIS:

Among hospital acquired infections candidiasis is graded on fifth position and third to fourth among blood related nosocomial infections globally ^(19, 20). It can invade inside the body and may infect our blood stream, body fluids like peritoneal, pleural, synovial, biliary fluids ⁽²¹⁾. Although globally *C. albicans* are the major threat but now trends are changing and non *albicans* (NCACs); mainly *C. glabrata* which is responsible for nosocomial blood stream infections ranging from 18-25% as reported from the year 1992 to 2007 ⁽²²⁾.

Every year 159,253 cases of candidemia are reported across the globe most admitted in hospital ICU, oncology and transplant units. The recent report of WHO is highly alarming stating that Pakistan is the country with the highest incidence of candidemia, which is in contrast to other countries have 38,795 cases (21 cases/100,000 people) per year ⁽²³⁾.

Among invasive fungal infections, candida was found to be 97.9% responsible agent in the year 2006 to 2009 in a tertiary care lab of Karachi. The main species responsible was *C. tropicalis* 32 - 38%, followed by *albicans* 20-21% (21, 24, 25), while two

other studies reported death rates due to candidemia ranged from 23 - 52% in Karachi ^(10, 21, 26).

Neonatal invasive candidiasis: is common in infants who are born with very low birth weights (< 1500) ⁽²⁷⁾. It is considered as the third frequent (10-12%) cause of septic infection in neonates admitted in NICU ⁽²⁸⁾. Regarding neonatal candidiasis, limited data is available to estimate the magnitude of the problem. A study conducted by Ariff et al (2011), in a hospital setting of Karachi showed *C. albicans* was the main culprit (55%) followed by *C. tropicalis* (21%) Karachi ⁽²⁹⁾. According to two other studies, the death rate of ICU neonates due to candidiasis was 24 -75% ^(28, 30) which is high in comparison with data available from United States, that is 20- 30% ^(20, 27).

NON INVASIVE CANDIDIASIS:

Candidiasis of mucosa is a frequent and recurrently occurring infection, affecting healthy as well as immunocompromised individuals. As it is a normal commensal resident of skin and mucosal epithelium of genitourinary areas, oral and digestive tract ⁽³¹⁾, therefore easily infect these areas under propitious circumstances.

No any significant study still shows the agreement of prevalence of oral candidiasis in Pakistan except one which showed positive association of 36% to 40% candidal carriage in betel quid and gutka habitual consumers in comparison with non-chewing controls ⁽³²⁾.

The occurrence of esophageal candidiasis according to studies in few settings of Pakistan calculated was 14% to 33% ⁽¹⁰⁾. According to a cross sectional study with 95% confidence interval *Candida tropicalis* was found responsible for blood stream candidal infection due to GI candidiasis ⁽²¹⁾.

Now we come to the candidiasis of genitourinary tract, it is a common site candidal colonization as normal resident. Candidiasis of reproductive tract is assumed on second position as the frequent cause of vaginal infection. About 75% of females faces the problem of vulvovaginitis due to candida once in their life with the recurrence rate of 5-10% overall ⁽³³⁾. In perception of Pakistan it was found 38% frequent among pregnant females who reported in an institutional hospital-based setting and its recurrence rate reported recently by another study setting was 50.6% ^(10, 33).

ROLE OF ANTIFUNGALS IN CANDIDIASIS:

Despite that candidal infections are highly prevalent, WHO says that there is great lacking in availability of substantial data and national survey programs on antifungal susceptibility pattern in the regions of Asia and south east America ⁽³⁴⁾. However,

studies in different clinical set-ups across the globe are available to estimate the resistance patterns of antifungal drugs.

Since many decades the polyenes mainly amphotericin B and nystatin were used commonly for the treatment of candidiasis. These drugs have fungicidal action but are highly nephrotoxic which limits their oral use, moreover, its new lipid oral formulation is not cost effective, reachable to all areas of Pakistan and affordable to every socioeconomic background patient for effective prolonged therapy. Among azoles, another antifungal class, acts as fungistatic. Fluconazole is one of this, commonly used as drug of choice against candidiasis these days but the gradual rising of reports of antifungals compromised sensitivity since 1990 around the world, mainly by non albicans that are *C. glabrata*, *C. krusei* and *C. auris* species, due to few available antifungal drug categories availability ^(17, 34, 35). According to WHO along with the underdeveloped countries there are some areas of developing world, where the patients don't have access to all antifungal drugs. The only available agent is fluconazole, which is already resistant to certain candida species and also carries cross resistance against other azoles, leaving the patient with no other effective treatment in those regions of the world ⁽³⁴⁾.

To estimate the magnitude of prevalence of candidal infections and the increasing resistance and cross resistance of candidal infection against antifungals in Pakistan, the data based on various clinical set-ups is given in table 3 and 4, besides for comparison global data is mentioned below in table 2 ⁽³⁶⁾. In these studies *Candida glabrata* was found to be more resistant to fluconazole in comparison with other antifungals as it is a commonly used antifungal for therapy and prophylaxis globally ^(23, 37). Though the most resistant specie found in the continent of Asia was *C. tropicalis* ranging from 0 – 83% ⁽³⁷⁾.

Table 2: Multicentered global antifungal surveillance studies showing resistance pattern to antifungals against candidiasis. ⁽³⁶⁾

	Study:	Most resistant specie isolated	Rise in resistance antifungals (%)	Duration of study
1.	ARTEMIS global Antifungal Surveillance, 2007.	<i>C. glabrata</i>	18 - 25	1992 -2007
2.	CANDIPOP study	<i>C. glabrata</i>	8-13.2	2002-2011

Table: 3:Over all and individual resistance patterns antifungals of candidal isolates.

	Author and year	Samples from	Tertiary settings	Antifungal drugs	No. of candidal isolates	Overall resistance (%)	Resistance (%)	Most resistant specie/s isolated
1	Jamil et al., 2017 ⁽³¹⁾	Candidal Renal transplant patients.		Fluconazole	164	34%	85%	
				Amphotericin -B			7%	
2	Khan and Baqai, 2010. ⁽³⁸⁾	Vulvovaginal candidal fertile females	Different Hospitals of Karachi.	Clotrimazole	100	40%	30%	
				Fluconazole			67%	
				Nystatin			38%	
3	Tasneem et al., 2017. ⁽³⁵⁾	All in and out candidal patients	Quaid-e-Azam Int. Hospital, Islamabad.	Amphotericin B	219		3%	C. albicans (2.3%)
				Fluconazole	219		15%	C. krusei (24%)
				Voriconazole	219		18%	C. parapsilosis (33%)
4	Zafar et al., 2015. ⁽³⁹⁾	Sexually active, vaginal candidal female patients.	Khyber Teaching Hospital, Peshawar.	Clotrimazole	40	61.3%	28%	C. glabrata (69%)
				Ketoconazole			70%	C. albicans (86%)
				Fluconazole			73%	C. albicans (76%)
				Itraconazole			75%	C. glabrata (100%) C. krusei (100%)

Table: 4:Cross resistance of antifungals among candidal isolates:

	Author and year	Samples from	Tertiary settings	Antifungals	Cross resistance in %	Most resistant specie/s isolated
1.	Jamil et al., 2017. ⁽¹⁶⁾	Candidal Renal transplant patients.	Kidney Centre Al-Sayyed hospital, Rawalpindi.	Fluconazole, Voriconazole, Ketoconazole	25%	
				Fluconazole, Ketoconazole	75%	
				Fluconazole, Amphotericin B	4.9%	
2.	Tasneem et al., 2017. ⁽¹⁷⁾	All in and out candidal patients	Quaid-e-Azam Int. Hospital, Islamabad.	Fluconazole, Voriconazole,	8.2%	C. albicans (88%), C. glabrata (11%)
				Amphotericin B, Voriconazole	6.3%	C. glabrata (6%)
				Fluconazole, Amphotericin B	6.3%	C. albicans (6%)

CONCLUSION

Resistance to antifungal drugs is heading as one of the threat towards the prompt and effective treatment of candidiasis. The most possible cause of rising resistance in our clinical set-up is irrational and repeated use of these agents and self-medication. Most of the time, antifungal drugs are prescribed to the candidal patients blindly, that results in the treatment failure being infected with the resistant specie. Subsequently, these agents are prescribed repeatedly and for prolong duration with lack of patients' compliance which causes recurrence of the infection with increased drug resistance. Most of the patients have comorbid and are already taking multiple drugs which causes increased drug-drug interactions with antifungals resulting in their treatment failure or increased toxic effects of the drugs. Furthermore, repeated and prolong use of the antifungal drugs which are usually very expensive lead to financial burden on majority of our low socioeconomic patients and the reason for abandonment of their treatment before the recommended period.

RECOMMENDATIONS

To minimize the resistance against antifungal drugs in our clinical set-up the clinicians should move one step ahead by recommending culture and sensitivity (C/S) for fungal organisms as a routine lab test to identify the specie responsible for infecting the patient and on this basis, the clinicians should prescribe more sensitive antifungal drug accordingly. This will have profound and promising results in achieving better therapeutic responses. Furthermore, this will cut down their expenses as well as help to minimize the prevalence of resistant candida species in our clinical set-up. Since, candidal infected patients are usually immuno-compromised, have comorbid and generalized health problems, therefore a more effective antifungal drugs prescription based on C/S will also help to improve their generalized health. Nevertheless, in the current scenario it is mandatory to explore newer antifungal drugs or alternative agents, with less side effects, drug-drug interactions and have low cost to get more treatment options.

Preventive steps should also be taken at national level to initiate properly managed surveillance programs to control spread of candidal infections especially in hospitals, and public places situated in the basements (without proper sunlight). Hospitals should be properly fumigated specific for fungi at frequent intervals to get rid-off these nosocomial organisms.

REFERENCES

1. Garcia-Solache MA, Casadevall A. Global warming will bring new fungal diseases for mammals. *MBio*. 2010;1(1):e00061-10.
2. Branquinho MH, Kneip LF. Fungal infections: a veritable worldwide problem. *Journal of Infectious Diseases and Medical Microbiology*. 2017;1(1).
3. Köhler JR, Casadevall A, Perfect J. The spectrum of fungi that infects humans. *Cold Spring Harbor perspectives in medicine*. 2015;5(1):a019273.
4. Pinalto KM, Alspaugh JA. New horizons in antifungal therapy. *Journal of Fungi*. 2016;2(4):26.
5. Salma S, Shah M, Rehman S. Rainfall trends in different climate zones of Pakistan. *Pakistan Journal of Meteorology*. 2012;9(17).
6. Negri M, Salci TP, Shinobu-Mesquita CS, Capoci IR, Svidzinski TI, Kioshima ES. Early state research on antifungal natural products. *Molecules*. 2014;19(3):2925-56.
7. Richardson M, Lass-Flörl C. Changing epidemiology of systemic fungal infections. *Clinical Microbiology and Infection*. 2008;14(s4):5-24.
8. Martins N, Ferreira IC, Barros L, Silva S, Henriques M. Candidiasis: predisposing factors, prevention, diagnosis and alternative treatment. *Mycopathologia*. 2014;177(5-6):223-40.
9. Butts A, Krysan DJ. Antifungal drug discovery: something old and something new. *PLoS pathogens*. 2012;8(9):e1002870.
10. Jabeen K, Farooqi J, Mirza S, Denning D, Zafar A. Serious fungal infections in Pakistan. *European Journal of Clinical Microbiology & Infectious Diseases*. 2017;36(6):949-56.
11. Kabir MA, Ahmad Z. Candida infections and their prevention. *ISRN preventive medicine*. 2012;2013.
12. Sanguinetti M, Posteraro B, Lass-Flörl C. Antifungal drug resistance among Candida species: mechanisms and clinical impact. *Mycoses*. 2015;58(S2):2-13.
13. Aslam A, Akhtar N, Hasan F, Shah AA. Prevalence and in vitro Antifungal Susceptibility Pattern of Candida species in a Tertiary Care Hospital, Rawalpindi, Pakistan. *Pakistan J Zool*. 2015;47(2):335-42.
14. Rashid A, Irum A, Malik IA, Ashraf A, Rongqiong L, Liu G, et al. Ecological footprint of Rawalpindi; Pakistan's first footprint analysis from urbanization perspective. *Journal of Cleaner Production*. 2018;170:362-8.
15. Khan F, Amatya B, Sayed TM, Butt AW, Jamil K, Iqbal W, et al. World Health Organization Global Disability Action Plan 2014–2021: Challenges and Perspectives for Physical Medicine and Rehabilitation in Pakistan. *Journal of rehabilitation medicine*. 2017;49(1):10-21.
16. Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC. Hidden killers: human fungal infections. *Science translational medicine*.

2012;4(165):165rv13-rv13.

17. Tasneem U, Siddiqui MT, Faryal R, Shah AA. Prevalence and antifungal susceptibility of *Candida* species in a tertiary care hospital in Islamabad, Pakistan. *JPMA The Journal of the Pakistan Medical Association*. 2017;67(7):986-91.
18. Khanum I, Mahmood SF, Waqar H, Awan S. Clinical Characteristics and Risk Factors of Candidemia in Tertiary Care Hospital. *Infectious Diseases Journal of Pakistan*. 2015;24(3):859.
19. Yapar N. Epidemiology and risk factors for invasive candidiasis. *Therapeutics and clinical risk management*. 2014;10:95.
20. Uppuluri P, Khan A, Edwards JE. Current Trends in Candidiasis. *Candida albicans: Cellular and Molecular Biology*: Springer; 2017. p. 5-23.
21. Farooqi J, Jabeen K, Saeed N, Iqbal N, Malik B, Lockhart S, et al. Invasive candidiasis in Pakistan: clinical characteristics, species distribution and antifungal susceptibility. *Journal of medical microbiology*. 2013;62(2):259-68.
22. Pfaller M, Castanheira M, Lockhart S, Ahlquist A, Messer S, Jones R. Frequency of decreased susceptibility and resistance to echinocandins among fluconazole-resistant bloodstream isolates of *Candida glabrata*. *Journal of clinical microbiology*. 2012;50(4):1199-203.
23. Bongomin F, Gago S, Oladele RO, Denning DW. Global and Multi-National Prevalence of Fungal Diseases—Estimate Precision. *Journal of Fungi*. 2017;3(4):57.
24. Farooqi J, Jabeen K, Saeed N, Zafar A, Brandt ME, Hasan R. Species identification of invasive yeasts including *Candida* in Pakistan: limitations of phenotypic methods. *JPMA The Journal of the Pakistan Medical Association*. 2012;62(10):995.
25. Sanglard D. Emerging threats in antifungal-resistant fungal pathogens. *Frontiers in medicine*. 2016;3:11.
26. Kumar S, Kalam K, Ali S, Siddiqi S, Baqi S. Frequency, clinical presentation and microbiological spectrum of candidemia in a tertiary care center in Karachi, Pakistan. *Age*. 2014;35:13-27.
27. Botero-Calderon L, Benjamin Jr DK, Cohen-Wolkowicz M. Advances in the treatment of invasive neonatal candidiasis. *Expert opinion on pharmacotherapy*. 2015;16(7):1035-48.
28. Khan EA, Choudhry S, Fatima M, Batool Z. Clinical spectrum, management and outcome of neonatal candidiasis. *risk*. 2015;19:38.8.
29. Ariff S, Saleem AF, Soofi SB, Sajjad R. Clinical spectrum and outcomes of neonatal candidiasis in a tertiary care hospital in Karachi, Pakistan. *The Journal of Infection in Developing Countries*. 2011;5(03):216-23.
30. Kaur H, Chakrabarti A. Strategies to Reduce Mortality in Adult and Neonatal Candidemia in Developing Countries. *Journal of Fungi*. 2017;3(3):41.
31. Jamil B, Bokhari MTM, Saeed A, Bokhari MZM, Hussain Z, Khalid T, et al. Candidiasis: Prevalence and resistance profiling in a tertiary care hospital of Pakistan. *JPMA The Journal of the Pakistan Medical Association*. 2017;67(5):688.
32. Abduljabbar T, Hussain M, Adnan T, Vohra F, Javed F. Comparison of oral *Candida* species prevalence and carriage among gutka-chewers and betel-quid chewers. *JPMA The Journal of the Pakistan Medical Association*. 2017;67(3):350-4.
33. Parveen N, Munir AA, Din I, Majeed R. Frequency of vaginal candidiasis in pregnant women attending routine antenatal clinic. *Journal of the College of Physicians and Surgeons--Pakistan: JCPSP*. 2008;18(3):154-7.
34. Organization WH. Antimicrobial resistance: global report on surveillance: World Health Organization; 2014.
35. Kontoyiannis DP. Antifungal Resistance: An Emerging Reality and A Global Challenge. *The Journal of infectious diseases*. 2017;216(suppl_3):S431-S5.
36. Perlín DS, Rautemaa-Richardson R, Alastruey-Izquierdo A. The global problem of antifungal resistance: prevalence, mechanisms, and management. *The Lancet Infectious Diseases*. 2017.
37. Whaley SG, Berkow EL, Rybak JM, Nishimoto AT, Barker KS, Rogers PD. Azole antifungal resistance in *Candida albicans* and emerging non-*albicans* *Candida* species. *Frontiers in microbiology*. 2017;7:2173.
38. Khan F, Baqai R. In vitro antifungal sensitivity of fluconazole, clotrimazole and nystatin against vaginal candidiasis in females of childbearing age. *Journal of Ayub Medical College Abbottabad*. 2010;22(4):197-200.
39. Zafar S, Khurram M, Usman R, Khan F, Nasim R. Clotrimazole, fluconazole, ketoconazole and itraconazole susceptibilities of *Candida* species in vulvovaginitis. *Cell*. 2015;342:9766463.