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Prevalence of Azole Resistance in Various Candida Species in Various Sample Along with Its antifungal Susceptibility Pattern in a Tertiary Care teaching hospital rural Gujarat, Vadodara.

Dr Binda Pipaliya[1], Dr Saurabh Chhotalal Norris[2], Dr Himani Pandya [3], Dr Tanuja Javadekar[4]

[1]Assistant Professor, Microbiology Department, Department of Microbiology, Smt. B. K. Shah Medical Institute & Research Centre, Sumandeep Vidyapeeth deemed to be University, Piparia, Ta Waghodia, Dist- Vadodara, Gujarat -391760, India.

[2]Assistant Professor, Microbiology Department, Department of Microbiology, Smt. B. K. Shah Medical Institute & Research Centre, Sumandeep Vidyapeeth deemed to be University, Piparia, Ta Waghodia, Dist- Vadodara, Gujarat -391760, India.

[3]Associate Professor, Department of Microbiology, Smt. B. K. Shah Medical Institute & Research Centre, Sumandeep Vidyapeeth deemed to be University, Piparia, Ta Waghodia, Dist- Vadodara, Gujarat -391760, India.

[4]Professor & Head, Department of Microbiology, Smt. B. K. Shah Medical Institute & Research Centre, Sumandeep Vidyapeeth deemed to be University, Piparia, Ta Waghodia, Dist- Vadodara, Gujarat -391760, India.

Author of correspondence: Dr.Saurabh Chhotalal Norris

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Abstract

Introduction: Candida is a type of yeast or fungus that normally resides in small amounts in the human body, particularly in the mouth, throat, gut, and vagina, without causing any harm. However, under certain conditions, such as a weakened immune system or disruption of the normal balance of microorganisms in the body (such as through antibiotic use), Candida can overgrow and cause infections. The in vitro susceptibility testing of antifungal agents is becoming increasingly important because of the introduction of new antifungal agents and the recovery of clinical isolates that exhibit inherent or developed resistance to Antifungal agents. Antifungal drug resistance in Candida species is a growing concern, particularly in healthcare settings where these infections can be challenging to treat. An antifungal agent is a drug that selectively eliminates fungal pathogens from a host with minimal toxicity to the host. Fluconazole, itraconazole, and ketoconazole inhibit cytochrome P_{450}-dependent enzymes (particularly C14-demethylase) involved in the biosynthesis of ergosterol, which is required for fungal cell membrane structure and function.

Aims and Objectives: The purpose of this study was to determine the prevalence of Azole resistance in various *Candida species* and its antifungal susceptibility profile in a tertiary care teaching hospital of rural Gujarat, India.

Materials and Methods: This is an observational retrospective study. Data was collected for a period of five years (from January 2016 to December 2020) using Laboratory Information System (LIS) of Central Diagnostic laboratory of a tertiary care teaching hospital in rural Gujarat. The antifungal tested were Clotrimazole, Fluconazole, Voriconazole with isolated fungal species in our study.

Results: A total of 53,195 culture samples were received during the study period with culture positivity rate of 37.27% (n=19,829) including bacterial and fungal isolates. Out of 19,829 isolates, 544 (2.74%) belonged to *Candida species.* Majority of *candida* isolates i.e., 70.8% were from critical care units. The most common species isolated was *Candida tropicalis* (44.30%) followed by *Candida albicans* (36.02%), *Candida glabrata* (8.08%) and *Candida parapsilosis* (4.59 %). The drug susceptibility of *Candida tropicalis* to Fluconazole, Voriconazole were 91%,95.3% respectively. Percentage susceptibility of *Candida albicans*, ranged from 85.7% to 99.4% for various antifungal agents. The susceptibility rates for *Candida glabrata* ranged from 34.7% to 100% for all antifungal agents tested. The susceptibility rates for *Candida parapsilosis* were 42.8% to 100% for various antifungal agents tested.

Conclusion: Majority of the infections were found in ICUs. The changing epidemiology of Candidiasis, therefore highlights the need for close monitoring of Candida species distribution and susceptibility to optimize therapy and outcome. We should also develop the guideline for empirical therapy based on epidemiology of India. Candida non-albicans species were more resistant to azoles compared to C. albicans, information that can be useful for clinicians dealing with non - responding cases.

Keywords: Candida albicans, Candida non-albicans, Antifungal Susceptibility Pattern.

Introduction

Candidiasis is the commonest fungal disease affecting mucosa, skin, nails, and internal organs. Itis caused by various species of yeast like fungi belonging to genus Candida. The infection may be acute or chronic, superficial, or deep and its clinical spectrum is wide. It is found mainly as secondary infection in individuals with some underlying immunocompromised condition and very rarely as the primary disease.[1]

Candida species was initially known only to be a part of normal flora of humans, however due to certain factors, the incidence of pathogenic Candida species have been increased. The various predisposing factors which precipitate these infections are:

• Natural receptive states like infancy, old age, pregnancy.
• Changes in local bacterial flora secondary to antibiotics.
• Changes in epithelial surface due to moisture, trauma.
• Primary or Secondary T-lymphocytes defects due to underlying disease e.g immunosuppression or AIDS.
• Endocrine diseases like Diabetes Mellitus
• Miscellaneous condition like Zinc or Iron deficiency.[1]

Candida species are responsible for causing infections in immunocompetent hosts as well. An increasing variety of *Candida spp* with resistance to antifungal drugs has also been documented in recent past. *Candida species* such as *C. tropicalis, C. krusei, C. glabrata, and C. parapsilosis* are found to be less susceptible to azole group of antifungal agents and create therapeutic challenge. Resistance can develop through several mechanisms:

1. **Intrinsic Resistance**: Some Candida species inherently have reduced susceptibility to certain antifungal drugs. For example, Candida krusei is naturally less susceptible to fluconazole.
2. **Acquired Resistance**: This occurs when Candida strains that were previously susceptible to antifungal drugs develop resistance over time due to genetic mutations or other mechanisms.
3. **Biofilm Formation**: Candida species can form biofilms on surfaces like medical devices (e.g., catheters), which provide a protective environment and can reduce the effectiveness of antifungal treatments.
4. **Overuse or Misuse of Antifungal Agents**: Excessive or inappropriate use of antifungal drugs can select for resistant Candida strains. This includes prolonged or repeated courses of antifungal therapy.
5. **Cross-resistance**: Resistance to one class of antifungal drugs (e.g., azoles) can sometimes confer resistance to other classes (e.g., echinocandins).

Managing antifungal drug resistance in Candida infections involves several strategies:

- **Antifungal Susceptibility Testing**: This helps guide treatment by identifying the most effective antifungal drug for the specific Candida strain causing the infection.

- **Combination Therapy**: In some cases, using two or more antifungal agents with different mechanisms of action can enhance effectiveness and reduce the likelihood of resistance development.
- **Infection Control Practices**: Strict adherence to infection prevention and control measures can reduce the incidence of Candida infections, thereby limiting the need for antifungal therapy.
- **Antifungal Stewardship**: Promoting appropriate use of antifungal drugs, including proper dosing and duration of therapy, helps minimize selective pressure for resistance.

Research continues into new antifungal agents and alternative treatments to address resistant Candida infections. Surveillance of antifungal resistance patterns is also important to monitor trends and inform treatment guidelines. The commonly used antifungal drugs show significant variation in the susceptibility pattern among the types of Candida species. The drug resistance scenario has been increasing during last decades due to over growing use of random antifungal agents.[2]

Candida albicans accounts for 40-60% isolated in developed countries[3] whereas in Indian report show increased predominance of Candida non albicans isolates.[4]Study from India has shown very high resistance to voriconazole and fluconazole. Some study from India have shown that the Candida albicans isolates were 100% susceptible to Amphotericin B and flucytosine and Certain species like Candida krusei and Candida glabrata had shown high degree of resistance to Fluconazole[5,6]

Therefore, the species identification of the Candida isolates, along with their anti-fungal susceptibility patterns can influence the treatment options for the clinician and have a beneficial impact on the patient care.

MATERIALS AND METHODS: This retrospective observational study was conducted at Shree Krishna Hospital, Karamsad, which is a tertiary care teaching hospital of Gujarat. The study included five years data from January 2016 to December 2020. The study was duly approved by Institutional Ethics Committee (IEC). A database of all cultures (bacterial or fungal) received during the study period was created by using Laboratory Information System of Central Diagnostic Laboratory at Shree Krishna Hospital, Karamsad. Details of fungal isolates and patient infected with them was collected with variables such as age, gender, site of infection e.g., respiratory tract, bloodstream, urinary tract, etc., and health care settings from where fungus was isolated (e.g., intensive care units,

trauma and emergency, wards of the patient). The isolates that were obtained in duplicate from the samples collected from the same site of infection were considered as a single isolate and were excluded from the study. *Candida species* and its antifungal susceptibility was studied further.

The identification and antifungal susceptibility of the *Candida species* were performed using automated system VITEK®2 Compact (bioMerieux, France) in Microbiology laboratory. Colony Morphology on Sabouraud's dextrose agar Candida produce creamy moist colony when incubated for 24-48 hours at 37°C. The isolates of Candida were confirmed by Gram stain, Germ tube formation, Color on CHROM agar Candida medium, Chlamydospore formation on Corn meal agar seen. [figure1,2] The antifungal agents tested included Clo-trimazole, Fluconazole, Voriconazole. Data was entered in Microsoft Excel and prevalence was calculated. Descriptive statistics was used to analyze data.

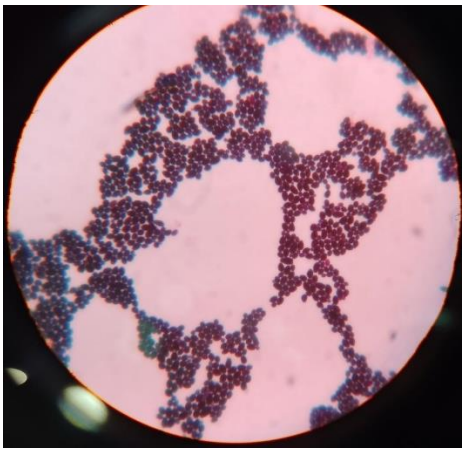


Figure 1: Gram Staining of candida

Figure 2. Colony of C. albicans on Sabouraud's Dextrose Agar

RESULTS: A total of 53,195 culture samples were received during the study with culture positivity of 37.27% (n=19,829) including bacterial and fungal isolates. Out of 19,829 isolates, 544 (2.74%) belonged to *Candida species*. Among the infected patient, 316 (58.0 %) were males and 228 (41.9%) were females. [Table/Fig-3] describes common clinical specimen from which *Candida species* were isolated. Out of 544 maximum 315 (57.9%) *Candida species* were isolated from urine specimen followed by 95 (17.4%) from blood specimen. Majority of *Candida spp.* i.e. 385 (70.7%) were from critical care units, followed by 159 (29.2%) from wards. Length of hospital stay of infected patients ranged from one day to 260 days with an average stay of 20 days. [Table/Fig-4] shows trend of prevalence of infections caused by *Candida species* isolated from 2016 to 2020, which was 3.28%, 2.61%, 2.39%, 2.25% and 3.57% in the years 2016, 2017, 2018, 2019 and 2020 respectively. *Candida tropicalis* was the most common C*andida species* followed by *Candida albicans*. [Table/Fig-5] Antifungal susceptibility of *Candida tropicalis* to Fluconazole & Voriconazole was 91.6% and 95.3 respectively[figure-6]

[Figure/Table-3] Clinical Specimen wise distribution of candida species.

Specimen	**Number of Isolates (%)**
Urine	315 (57.9%)
Blood	95 (17.4%)
Skin and Soft tissue	48 (8.8%)
Sputum	36 (6.6%)
Tracheal Secretion	29 (5.3%)
Bronchoalveolar lavage (BAL)	06 (1.1%)
Others	15 (2.7%)

[Table-1]: Clinical Specimen wise distribution of *Candida species* isolates.
'Others' include CVP tip, Pleural fluid, Stool, Peritoneal fluid, Oral scrapping

Figure-4

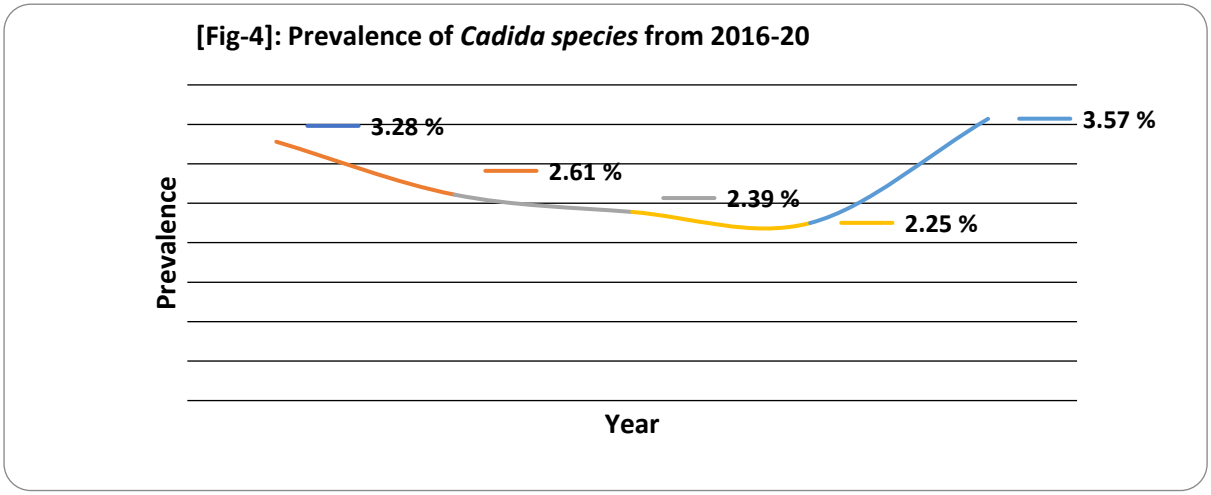
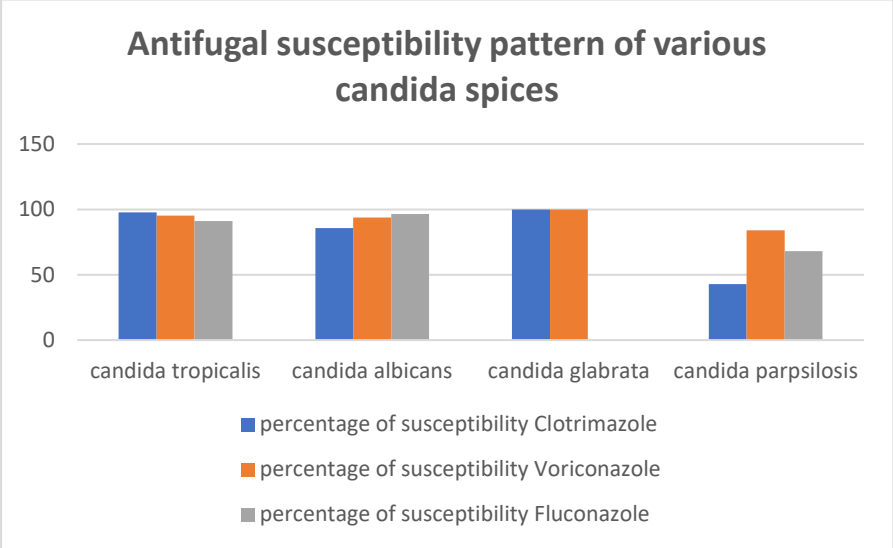


Figure-5

Candida species	**Number of Isolates (%)**
Candida tropicalis	241 (44.3%)
Candida albicans	196 (36%)
Candida glabrata	44 (8%)
Candida parapsilosis	25 (4.5%)
Candida krusei	05 (0.9%)
Candida guilliermondii	04 (0.7%)
Candida famata	16 (2.9%)
Candida lusitaniae	06 (1.1%)
Candida kyfer	04 (0.7%)
Candida haemulonii	02 (0.3%)
Candida utilis	01 (0.1%)
Total	544

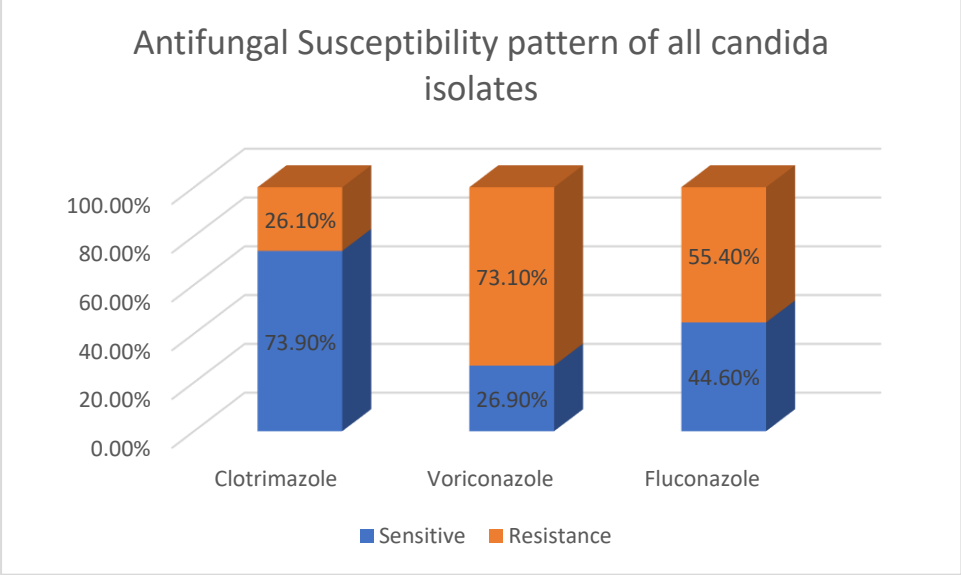
[Table-3]: Distribution of *Candida species* isolates.

Figure-6



Azole resistance in our study was highest in Clotrimazole (73.90%) followed by Fluconazole (44. 60%) and Voriconazole (26.90%). [figure-7].

Figure - 7



DISCUSSION: A total of 53,195 culture samples were received during the study period with culture positivity of 37.27% (19, 829) which includes the bacterial and fungal isolates. Out of 19, 829 isolates, 544 (2.74%) belonged to *Candida species.*

In our study, majority of *Candida species* were isolated from males i.e., 316 (58.0 %) similar to findings by R. Shukla from Telangana KIMS who found 67% male infected with candida in their study.[7]

We found that majority of *Candida species* infections were reported from critical care units i.e., 385 (70.7%), followed by 159 (29.2%) from wards. This was contrary to findings by Zhang from SouthWest China, who reported majority of *Candida* species from surgical wards (98, 42.4%) followed by medical wards (103,40.3%) and ICUs (42, 17.3%).[8]

Out of 544 *Candida species*, majority were isolated from urine specimen i.e., 315 (57.9%) followed by blood with 95 (17.4%) isolates like findings by Deorukhkar SC who reported 34.6% isolates from urine.[9] Similar to finding in other study, *Candida tropicalis* was most common candida species isolates in our hospital followed by *Candida albicans*.[10,11]

In contrast, in studies conducted by Western countries; Candida glabrata and Candida parapsilosis formed the predominant species .[12,13,14]

In our study, susceptibility rates of *Candida tropicalis* to Fluconazole and Voriconazole, were 91.6% and 95.3% respectively. These rates are higher than 65.2% and 82.6% reported for *Candida tropicalis* to Fluconazole & Voriconazole by R. Shukla from Telangana KIMS .[7]

Increasing Antifungal Drug Resistance in *Candida* species is a major threat in treating these infections. Resistance to Fluconazole is a great concern as it is the most common azole used for the treatment of disseminated candida infection. Fluconazole is available in both intravenous and oral formulations with high bioavailability and is more cost-effective than other antifungal agents. Although Amphotericin B is effective against most strains of *Candida* spp., it is not the first-line treatment for candidemia due to the nephron toxicity associated with it.[7,11] In our study Fluconazole is highly resistance in *Candida parapsilosis* (33.3%) followed by *Candida tropicalis* (7.2%) and *Candida albicans* (2.5%).

Conclusion

Candida species was initially known only to be a part of normal flora of humans. But the incidence of the Candida albicans and Candida non albicans as the pathogen came into picture. This Study highlights the emergence of non-albicans candida as major isolates and alarming Voriconazole resistance in C. non albicans. Azole antifungal drugs are commonly used to treat Candida infections, but the emergence of resistance poses a challenge in effectively managing these infections. It is crucial for healthcare professionals to monitor and address this issue through appropriate treatment strategies and surveillance measures to ensure optimal patient outcomes.

Proper Diagnosis: Ensuring accurate and timely diagnosis of Candida infections can help in selecting the most appropriate antifungal treatment, reducing the risk of resistance development. Rational Antifungal Use: Healthcare providers should prescribe antifungal medications judiciously, following guidelines and considering factors such as the type of infection, patient characteristics, and local resistance patterns. Combination Therapy: In some cases, using a combination of antifungal drugs with different mechanisms of action can help prevent or overcome resistance. Monitoring and Surveillance: Regular monitoring of antifungal resistance patterns in Candida species can help healthcare facilities adapt treatment protocols and infection control measures accordingly. Infection Control Practices: Implementing strict infection control measures, such as hand hygiene, environmental cleaning, and proper catheter care, can help prevent the spread of resistant Candida strains.

Conflict of interest: Nil

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