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# Molecular And Phenotypic Characterization Of Candida Species: Virulence Factors, Antifungal Susceptibility And Resistance Genes In Clinical Isolates

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## ABSTRACT

**Introduction:** The lack of effective diagnostics and treatments, along with rising antifungal resistance, exacerbates the problem. Candida species have emerged as a significant cause of opportunistic infections. Alongside the expression of various virulence factors, the rise of antifungal resistance among Candida species presents a considerable clinical challenge.

**Aim and Objective:** To study the molecular and phenotypic characterization of candida species its virulence factors, antifungal susceptibility, and resistance genes in clinical isolates

**Material and Methods:** A prospective observational study was conducted in the department of Microbiology. Candida species collected from patients received the department of Microbiology were processed. Identification was confirmed by standard microbiological techniques. Antifungal susceptibility testing was performed using CLSI guidelines. Phenotypic and Genotypic confirmation was carried. The Molecular characterization of resistance genes, CDR1, CDR2, MDR1 and ERG11 was performed using PCR assays where DNA Qiagen Extraction kit was used .

**Results:** In the present study the prevalence of the ERG11 gene was detected in 13.2% of 53 Candida albicans isolates, with MDR1, CDR1, and CDR2 genes present in 7.5%, 3.7%, and 1.8% of isolates, respectively. Among non-Candida strains, the ERG11 gene was identified in 10.3% of 58 Candida tropicalis isolates, while MDR1, CDR1, and CDR2 genes were found in 3.4%, 3.4%, and 1.7% of isolates, respectively.In the case of Candida krusei (n = 15), the ERG11 gene was prevalent in 13.3% of isolates, followed by MDR1, CDR1, and CDR2 genes at 13.3%, 0%, and 6.6%, respectively. Lastly, among Candida glabrata isolates (n = 7), the ERG11 gene was identified in 14.3% of isolates, with MDR1, CDR1, and CDR2 genes present in 14.3%, 0%, and 3.3% of isolates, respectively

**Conclusion:** Precise identification of virulence factors in Candida species is essential for predicting the response to antifungal therapy and identifying emerging strains with increased resistance. The coexistence of life-threatening invasive Candida infections and resistance to antifungal drugs is a global concern, making it crucial to understand the relationship between virulence factors and antifungal susceptibility in Candida.This highlights the need for targeted antifungal therapy and further research into the mechanisms linking virulence factors with drug resistance to improve the management of Candida infections.

***KEYWORDS:*** *Molecular, Phenotypic, Characterization, Candida Species, Virulence Factors, Antifungal Susceptibility, Resistance Genes.*

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## 1. INTRODUCTION

Candida species, particularly non-Candida albicans species (NCAS), have emerged as significant opportunistic pathogens, especially in immunocompromised patients and hospitalized individuals. Candida infections, ranging from superficial mucosal to life-threatening invasive candidiasis, are now considered a global health challenge due to rising antifungal resistance and evolving pathogenic mechanisms [1–3].

Fungal infections, such as candidiasis caused by *Candida*, pose a problem of growing medical concern. In developed countries, the incidence of *Candida* infections is increasing due to the higher survival of susceptible populations, such as immunocompromised patients or the elderly. Existing treatment options are limited to few antifungal drug families with efficacies that vary depending on the infecting species. In this context, the emergence and spread of resistant *Candida* isolates are being increasingly reported [4]. Understanding how resistance can evolve within naturally susceptible species is key to developing novel, more effective treatment strategies. However, in contrast to the situation of antibiotic resistance in bacteria, few studies have focused on the evolutionary mechanisms leading to drug resistance in fungal species.

Currently, there are only four major classes of antifungals in clinical use: azoles, polyenes, echinocandins, and pyrimidine analogs [5]. This situation alarmingly decreases the chances of a successful treatment and increases the possibilities of a fatal outcome if the infecting pathogen is resistant to one or multiple drugs. Limitations in diagnostic methods further enhance the problems of a few therapeutic options, as different species may show diverse resistance profiles. Thus, diagnostics of the infecting agent, along with susceptibility tests, should be used to inform the choice of therapy (discussed below). Over the last years, the intensive use of some antifungal drugs, such as azoles, has promoted a shift in the epidemiology of candidiasis, in which the incidence of *C. albicans* has decreased in favor of other species that are naturally less susceptible to this drug, such as *C. glabrata* [6].

The problem of the intrinsic variation of drug susceptibility among different *Candida,* we need to add the emerging issue of acquired resistance, which refers to the ability of yeasts to evolutionarily develop mechanisms that lower their susceptibility towards a given drug [7,8]. This process generally involves mutations ranging from chromosomal re-arrangements to point mutations. These mutations can affect drug resistance in different ways, ranging from directly interfering with the binding of the drug to its target to inducing gene expression changes that promote physiological states that reduce drug susceptibility. In this regard, an enhanced capacity to form biofilms can result in the acquisition of resistance, as these structures promote yeast survival upon exposure to the drug [9,10].

The emergence of resistant strains, including those becoming resistant to multiple drugs, has been increasingly reported in recent years. The azole drugs inhibit the ERG11 gene-derived L anosterol 14-α-demethylase enzyme involved in drug intake. It affects the biosynthesis of ergosterol and functions of drug efflux pumps Mdr1p and Cdr1p/Cdr2p. Echinocandins inhibit glycan synthesis. Polyenes bind with ergosterol and affect the efflux pump. Flucytosine inhibits the activity of DNA/RNA synthesis. Of the four classes of antifungal drugs, as the azole groups are used much, and drug resistance to azole drugs is high. In the case of azole-resistant, *C. albicans* the molecular level changes in drug resistance involve mutation of drug target genes ERG11 and ERG3and over-expression of ERG11, and up-regulation of drug efflux genes CDR1 and CDR2 [11]. In addition, it has been demonstrated that such resistant phenotypes can develop over the course of an infection, and in response to treatment, which adds yet another threat to patients. [12,13].

## 2. MATERIAL AND METHODS

A prospective observational study was conducted in the department of Microbiology. Candida species collected from patients received the department of Microbiology were processed.

Identification was confirmed by standard microbiological techniques. Antifungal susceptibility testing was performed using CLSI guidelines. Phenotypic and Genotypic confirmation was carried.

**Study Setting**
A total of 1130 clinical samples from various sources (urine, sputum, blood, vaginal swabs, pus, and endotracheal secretions) were included.

**Sample Collection and Culture**
Samples were processed using standard microbiological techniques. Sabouraud Dextrose Agar (SDA) was used for fungal culture, and incubated at 37°C for 48–72 hours. Identification of Candida species was performed using conventional methods (germ tube test, cornmeal agar morphology, sugar assimilation) and confirmed by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS).

**Virulence Factor Detection**
Biofilm formation was assessed using the tube method, while phospholipase activity was detected using egg yolk agar. Results were interpreted based on standard protocols.
**Antifungal Susceptibility Testing**

The Kirby-Bauer disk diffusion method was used on Mueller-Hinton agar supplemented with 2% glucose and 0.5 µg/mL methylene blue. Drugs tested included fluconazole, itraconazole, voriconazole, amphotericin-B, micafungin, nystatin, and cotrimoxazole.

**Molecular Analysis**
DNA extraction was done using a standard phenol-chloroform method. PCR was performed to detect ERG11, MDR1, CDR1, and CDR2 genes. Amplified products were analyzed by agarose gel electrophoresis.

**GENOTYPIC METHOD:**
**DNA Extraction :** For the detection of gene, chromosomal DNA from the clinical strains of *Candida species* was extracted. DNA extraction was carried out using a commercial available DNA extraction kit (Qiagen DNA Extraction Kit) as indicated by manufacturer's instructions. The extracted DNA was run in PCR for its extension according to standard method.

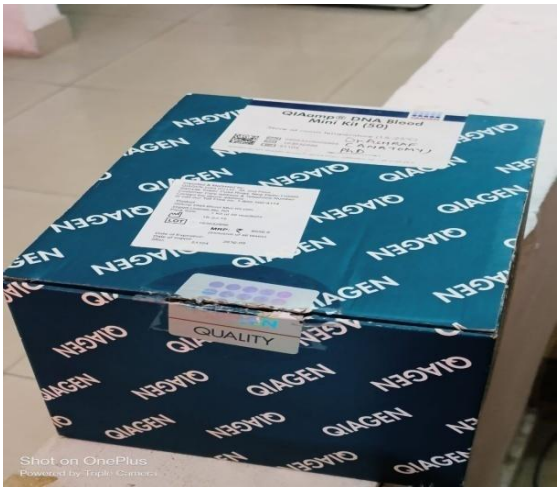



**Figure No. 1: The DNA Extraction kit**

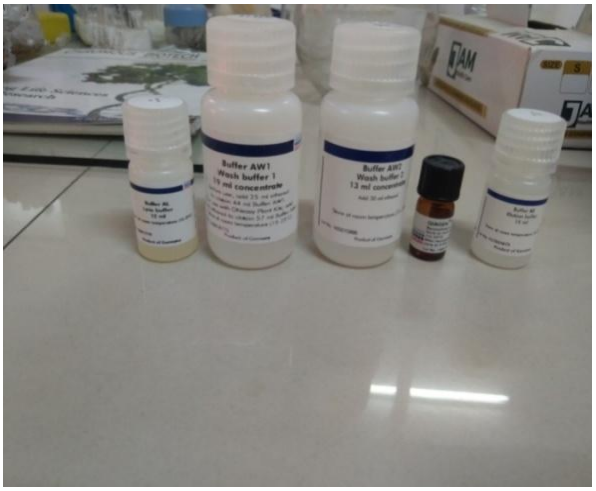



**Figure No. 2: The DNA Extraction Reagents**

**DNA extraction and PCR method**
Total genomic DNA was extracted from all fluconazole resistant positive isolates using a DNA extraction kit according to the manufacturer's instructions. Amplification and detection of the considered gene was done by the PCR method using specific primers.

**Table No. 1 :Primers used to amplify ERG11 gene fragments.**

| Fragment | Gene | Primer sequence | Length (bp) |
|---|---|---|---|
| A | ERG11-FA | 5'- ATGGCTATTGTTGAAACTGTC-3' | 785 bp |

| | | | |
|---|---|---|---|
| | ERG11-RA | 5'- CGTTCTCTTCTCAGTTTAATTTC-3' | |
| B | ERG11-FB<br>ERG11-RB | 5'- GAAGAGAACGTGGTGATATTGATC-3'<br>5'- CACTGAATCGAAAGAAAGTTGCC-3' | 826bp |

**Table No. 2 :Primers used to amplify CDR1 gene fragments.**

| Fragment | Gene | Primer sequence | Length (bp) |
|---|---|---|---|
| A | CDR1-FA<br>CDR1-RA | 5'CAATCACATTCGTCCTGGTTC-3'<br>3'-TTGAAAGCCAAGGACATCAC-5' | **387bp** |

**Table No. 3 :Primers used to amplify CDR2 gene fragments.**

| Fragment | Gene | Primer sequence | Length (bp) |
|---|---|---|---|
| A | CDR2-FA<br>CDR2-RA | 5'-TGGCAAACAATCCAACAATAC -3'<br>3'-AATCAAGGGAATAGATGGGTC-5' | 455bp |

**Table No. 4 :Primers used to amplify MDR1 gene fragments.**

| Fragment | Gene | Primer sequence | Length (bp) |
|---|---|---|---|
| A | MDR1-FA<br>MDR2-RA | 5'-ATGTTGGCATTCACCCTTC-3'<br>3'-GAAAACTTCTGGGAAAACTGG-5' | 426bp |

- The primers for  gene for the detection of gene of interest was designed and confirmed by NCBI [14].
- The primers were purchased from "**Saha gene'** and was reconstituted with sterile double distilled water based on the manufacturer's instruction.


**Figure No. 3:Primers for ERG11 gene**

**Figure No. 4: The Gel Documentation system**

The PCR tubes were transferred to the thermal cycler to start the amplification reaction according to specific program for pair of primers as in tables

**Polymerase Chain Reaction (PCR)**

The amplification of the ERG11 gene sequence was performed using PCR. Due to its length (1587 bp), the sequence was amplified in two fragments (A and B) using the primers shown in Table No. 1. The starters were designed using the Snap Gene program and the OligoAnalyzer tool and were synthesised by Genomed (Warsaw, Poland). The primers were designed to include a sequence of 20 bp downstream and 12 bp upstream (at positions 20 bp ERG11 or +1599 bp ERG11, respectively) to ensure the amplification of the entire ERG11 gene sequence [15].

**The PCR cycling conditions**

The PCRReactions were carried out in the following reaction mixture where 12.5 µLof the Master Mix (BioRad, Hercules, CA, USA), 0.5µ L of each of the 5 µ M primers, 2 µ L of isolated genomic DNA and 9.5 µ L of sterile water (total volume 25 µ L). Fragment "A" of the ERG11 gene sequence was amplified using a program called ZL-ERG11A and fragment "B" using the ZL-ERG11B program. The Thermal Cycler (BioRad, Hercules, CA, USA) was used to perform the PCR reaction. The PCR cycling conditions have been illustrated below shown in

| **Step** | **Program** | | | | **Cycles** |
|---|---|---|---|---|---|
| | **ZL-ERG11A** | | **ZL-ERG11B** | | |
| | **Time** | **Temperature** | **Time** | **Temperature** | |
| **Initial denaturation** | 5 min | 98 ºC | 5 min | 98ºC - | |
| **Denaturation** | 30 s | 98 ºC | 28 s | 98º C | 35 |
| **Annealing** | 30 s | 51 ºC | 29 s | 55 ºC | |
| **Extension** | 30 s | 72º C | 30 s | 72º C | |
| **Final extension** | 5 min | 72º C | 5 min | 72º C | |
| | ∞ | 4ºC | ∞ | 4ºC | |

**Table No. 5:The PCR cycling conditions to amplify ERG11 gene fragments.**

**The PCR cycling conditions to amplify ERG11 gene fragments.**

The Agarose Gel Electrophoresis was performed in order to identify the Purified PCR Product which was previously identified by its amplified DNA fragments. The resulting PCR product was subjected to 1 % agarose gel electrophoresis and visualized by Gel Doc™ EZ Gel Documentation System (Bio-Rad Laboratories Inc., Hercules, CA, USA). A 1 kb DNA Ladder (Thermo Fisher Scientific ™, Waltham, MA, USA) was used as the marker to evaluate the PCR product of the sample [15].

| **Step** | **Program** | | **Cycles** |
|---|---|---|---|
| | **CDR1** | | |
| | **Time** | **Temperature** | |
| **Initial denaturation** | 5 min | 98 ºC | |
| **Denaturation** | 30 s | 98 ºC | 35 |
| **Annealing** | 30 s | 51 ºC | |
| **Extension** | 30 s | 72º C | |
| **Final extension** | 5 min | 72º C | |
| | ∞ | 4ºC | |

**Table No. 6:The PCR cycling conditions to amplify CDR1 gene fragments.**

**The PCR cycling conditions to amplify CDR1 gene fragments.**

| Step | Program<br>CDR2<br>Time Temperature | Cycles |
|---|---|---|
| **Initial denaturation** | 5 min 98 ºC | |
| **Denaturation** | 30 s 98 ºC | 35 |
| **Annealing** | 30 s 51 ºC | |
| **Extension** | 30 s 72º C | |
| **Final extension** | 5 min 72º C | |
| | ∞ 4ºC | |

**Table No. 7:The PCR cycling conditions to amplify CDR2 gene fragments.**

| Step | Program<br>MDR1<br>Time Temperature | Cycles |
|---|---|---|
| **Initial denaturation** | 5 min 98 ºC | |
| **Denaturation** | 30 s 98 ºC | 35 |
| **Annealing** | 30 s 51 ºC | |
| **Extension** | 30 s 72º C | |
| **Final extension** | 5 min 72º C | |
| | ∞ 4ºC | |

**Table No. 8:The PCR cycling conditions to amplify MDR1 gene fragments.**

**Statistical Analysis**
Data were analyzed using SPSS version 25.0. Chi-square tests were used to compare proportions, and a p-value of <0.05 was considered statistically significant.

## 3. RESULTS

A total of 1130 subjects with similar demographic characteristics were included in the study, out of which 652 (57.6%) tested positive for culture while 478 (42.3%) tested negative for culture ($X^2$=26.7, *p*=0.0001). Among the culture positive samples (n=652), 514 (78.8%) were identified as GNB/GPC while 138 (21.1%) were classified as *Candida* species (X2=216.8, *p*=0.0004).

The identification of *Candida* species in the research involved two methods - the conventional method and the matrix-assisted laser desorption/ionization time-of-flight (MULTI-TOF) method. Out of the 138 *Candida* isolates, 38.4% were recognized as *Candida albicans* while the remaining 61.6% were grouped as non-*Candida albicans*. Within the non-*Candida albicans*category, *Candida tropicalis* was predominantly observed with 58 cases (68.2%), followed by *Candida krusei* with 15 cases (17.6%), *Candida glabrata* with 7 cases (8.23%), and an additional 5 cases (5.8%) of unidentified species. Both methods provided similar identification results for all isolates, with the exception of a particular *Candida* species. In the conventional method, it was identified as *Candida parapsilosis*, while in MULTI-TOF, it was labeled as *Candida metapsilosis*.

Further analysis showcases that the highest percentage of *C. albicans* was seen in sputum samples (39.6%) while a higher percentage of non-*Candida albicans* was noted in urine samples (44.7%).

Fungi possess certain factors that increase their ability to cause disease, including biofilm formation and phospholipid enzyme production. These factors help fungi to attach to host tissues, evade the immune response, and access nutrients. Biofilm formation creates a protective barrier that shields fungi from host defenses and antibiotics, aiding in their survival

and persistence in the host. On the other hand, phospholipid enzyme production allows fungi to breach host cell membranes, facilitating their spread within the host.

These virulence mechanisms are crucial for the development of fungal infections and contribute significantly to the overall pathogenicity of fungal pathogens. This study revealed that 53.5% of fungal isolates exhibited biofilm formation, with the majority belonging to *C. tropicalis* (58.03%). In contrast, only 10.04% of the isolates tested positive for Phospholipidase enzyme, with most identified as *C albicans* (11.6%).

Furthermore, when investigating the susceptibility of *Candida* isolates to antifungal drugs, it was found that Amphotericin-B had the highest sensitivity at 95%, followed by Voriconazole at 85% and itraconazole at 49.2%. Conversely, Micafungin was the least effective among the tested antibiotics.

Researchers across different countries have raised concerns about the increasing prevalence of antifungal-resistant Candida species, posing a significant challenge in clinical settings. Various studies conducted globally have revealed the underlying mechanisms of resistance in Candida species, including mutations in the ERG11 gene and the overlapping or upregulation of CDR1, CDR2, and MDR1 genes.

In a present study, the prevalence of the ERG11 gene was detected in 13.2% of 53 *Candida albicans* isolates, with MDR1, CDR1, and CDR2 genes present in 7.5%, 3.7%, and 1.8% of isolates, respectively. Among non-Candida strains, the ERG11 gene was identified in 10.3% of 58 *Candida tropicalis* isolates, while MDR1, CDR1, and CDR2 genes were found in 3.4%, 3.4%, and 1.7% of isolates, respectively.In the case of *Candida krusei* (n = 15), the ERG11 gene was prevalent in 13.3% of isolates, followed by MDR1, CDR1, and CDR2 genes at 13.3%, 0%, and 6.6%, respectively. Lastly, among *Candida glabrata* isolates (n = 7), the ERG11 gene was identified in 14.3% of isolates, with MDR1, CDR1, and CDR2 genes present in 14.3%, 0%, and 3.3% of isolates, respectively.

**Table 9: Frequency of culture positive and culture negative samples**

| | **Culture +ve (n,%)** | **Culture -ve (n,%)** | **$X^2$, p value** |
|---|---|---|---|
| TOTAL SAMPLE (n=1130) | 652 (57.6%) | 478 (42.3%) | 26.79, 0.0001* |
| Candida sp | 138 (21.1%) | | 216.8, 0.0004* |
| GNB/GPC | 514 (78.8%) | | |

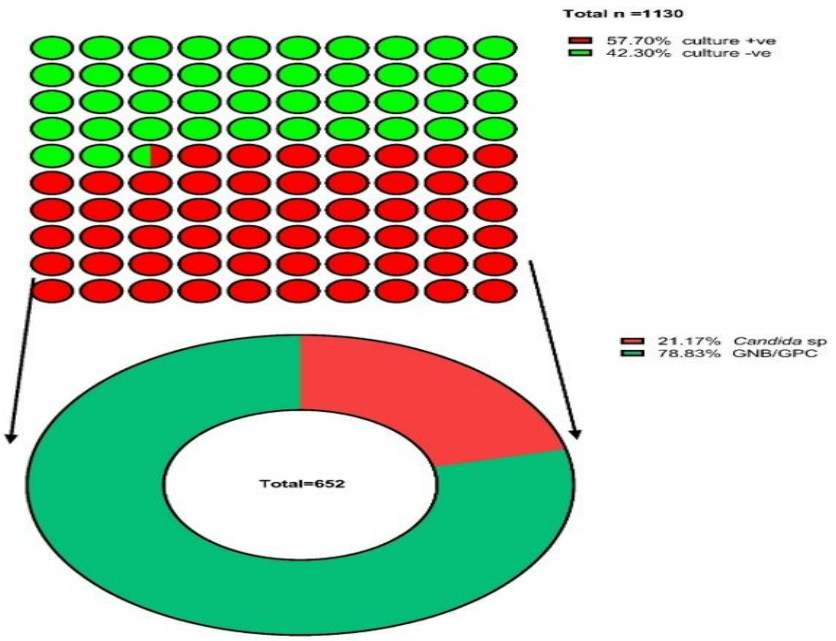



**Figure No. 4:Frequency distribution of culture positive and culture negative.**

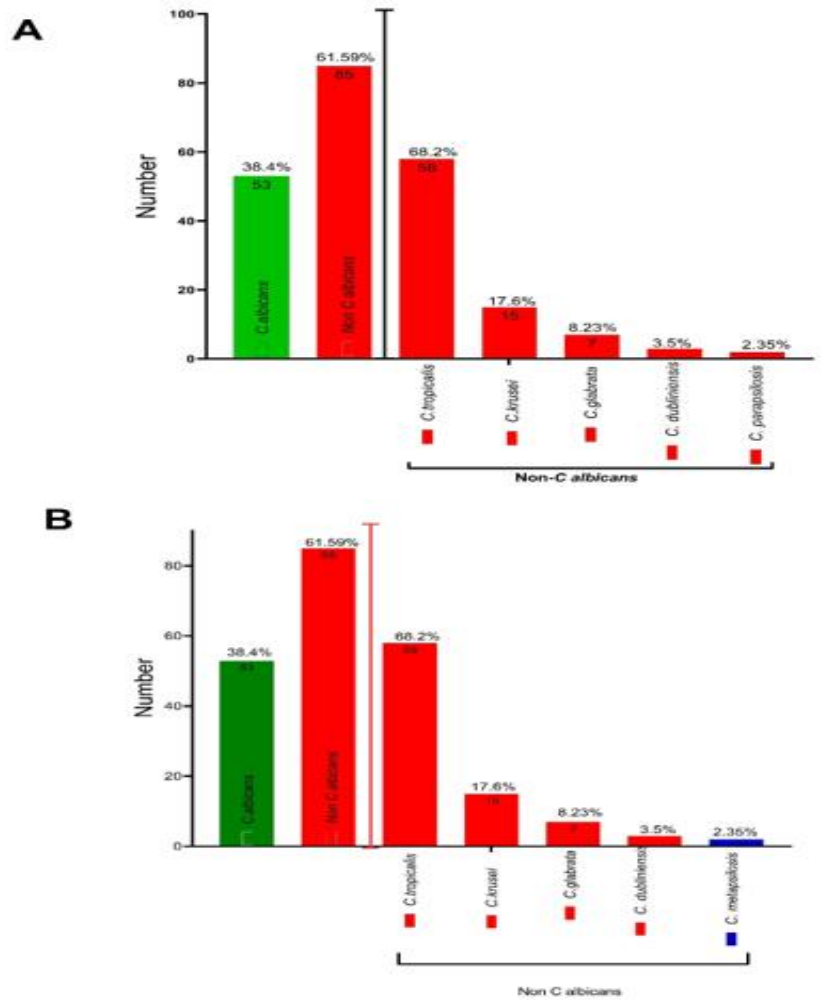



**Figure No. 5: Non alibicans**

**2:**Breakdown of *Candida* Samples and their respective proportions as determined by conventional (A) and MALDI-TOF (B) analysis.

**Table no. 10: Frequency of *C.albicans* & Non-Candida albicans among different samples.**

| SAMPLE | *C.albicans* (n=53) (n,%) | Non-Candida albicans (n=85) (n,%) | $X^2$, *p* value |
|---|---|---|---|
| Urine | 13 (24.5) | 38 (44.7) | 9.84, 0.079 |
| Pus | 3 (5.6) | 4 (7.07) | |
| Vaginal swab | 12 (22.6) | 19 (22.3) | |
| Blood | 2 (3.7) | 1 (1.17) | |

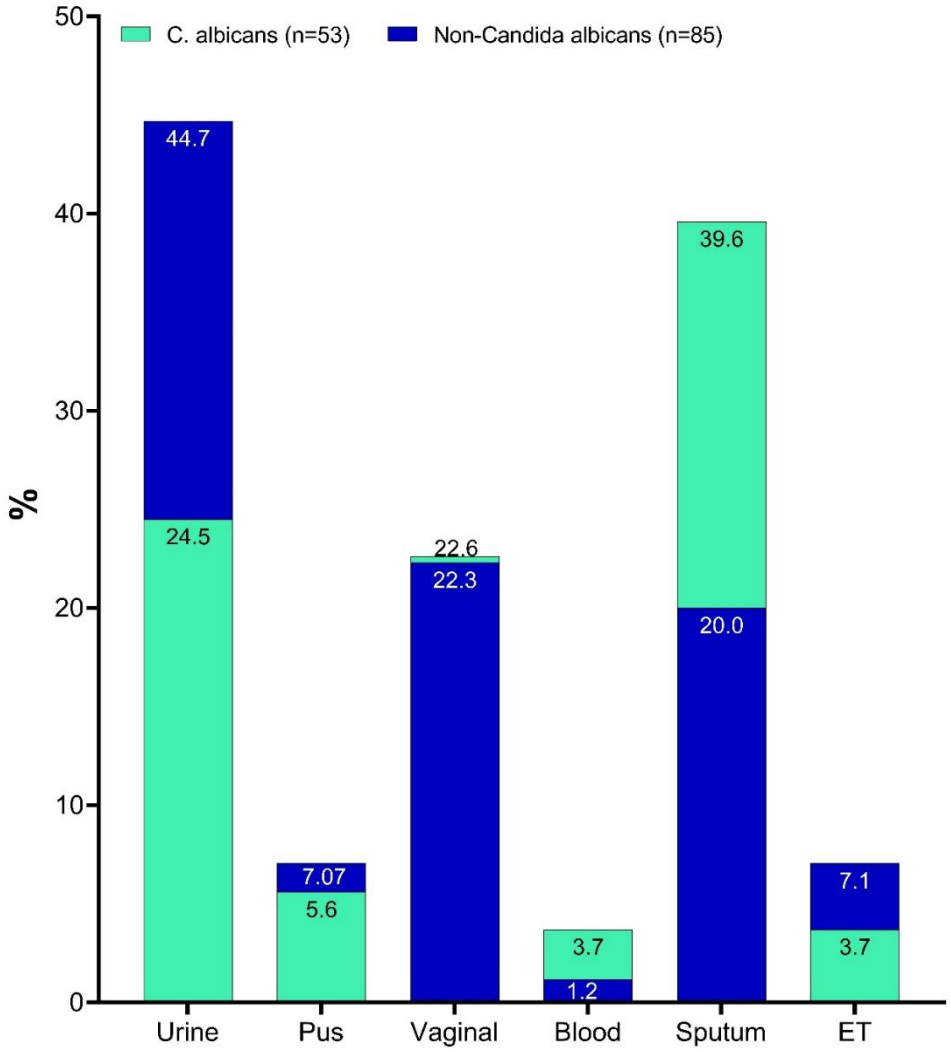



**Figure No. 6: Overlapping graph shows the Frequency distribution of *C.albicans* & Non-Candida albicans among different samples.**

**Table No. 11: Frequency of virulence factors among Various Candida isolates.**

| VIRULENCE FACTORS | Biofilm production (n,%) | Phospholipidase enzyme (n,%) |
|---|---|---|
| *C.albians* | 42 (37.5) | 16 (11.6) |
| *C.tropicalis* | 65 (58.03) | 3 (2.17) |
| *C.glabrata* | 2 (1.78) | 2 (1.44) |
| *C.krusei* | 3 (2.67) | - |

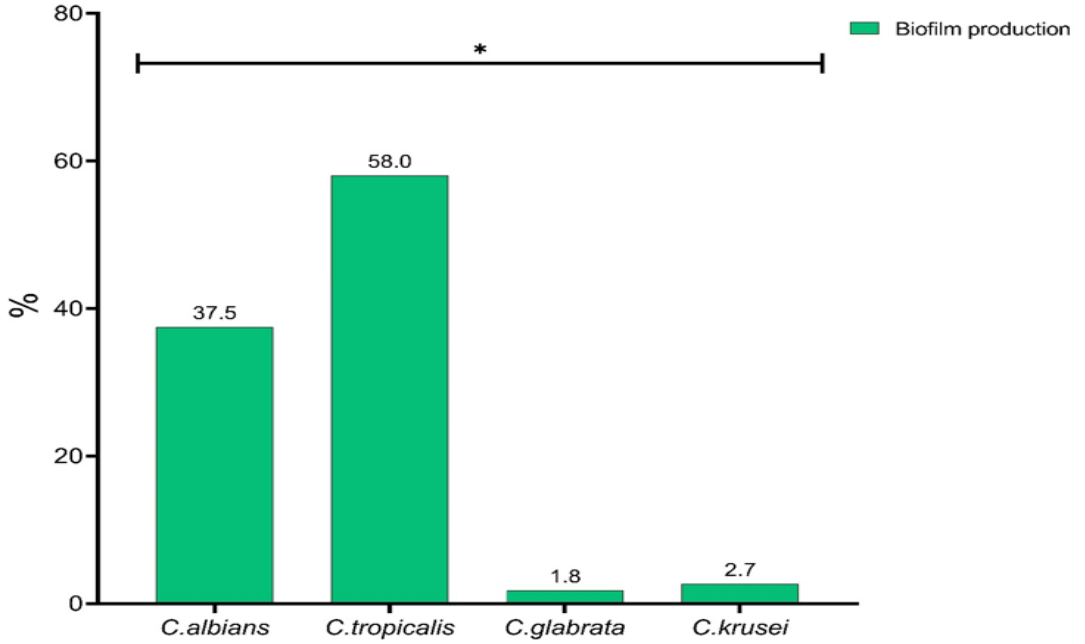



**Figure No.7:Incidence of biofilm formation among different Candida strains, expressed as a percentage.**

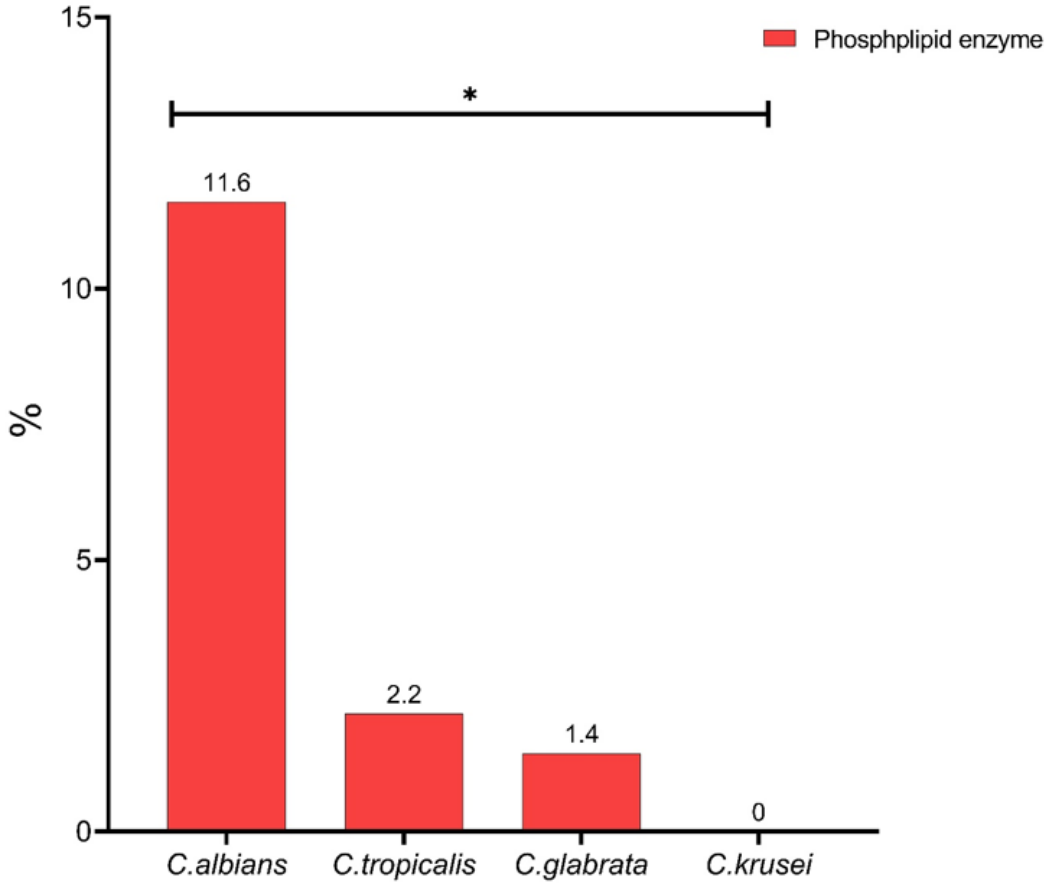



**Figure No.8 : Production of phospholipid enzyme among different Candida strains, expressed as a percentage of the total frequency.**

**Table No. 12 :Antifungal susceptibility of Candida isolates. (Kirby-bauer disk diffusion method)**

*S: sensitive; R: resistant*

| species / Antifungal Drug | Candida albicans(N=53) | | Non-candida albicans(N=85) | |
|---|---|---|---|---|
| | **S** | **R** | **S** | **R** |
| Fluconazole | 17(32.07%) | 36(67.92%) | 28(32.94%) | 57(67.05%) |
| Cotrimoxazole | 8(15.09%) | 45(84.90%) | 13(15.29%) | 72(84.70%) |
| Nystatin | 5(9.43%) | 48(90.56%) | 9(10.58%) | 76(89.41%) |
| Itraconazole | 31(58.49%) | 22(41.50%) | 37(43.52%) | 48(56.47%) |
| Voriconazole | 49(92.45%) | 4(7.54%) | 68(80%) | 17(20%) |
| Micafungin | 3(05.66%) | **50(94.33%)** | 5(5.88%) | **80(94.11)** |
| Amphotericin-B | 51(96.22%) | 2(3.77%) | 81(95.29%) | 4(4.70%) |

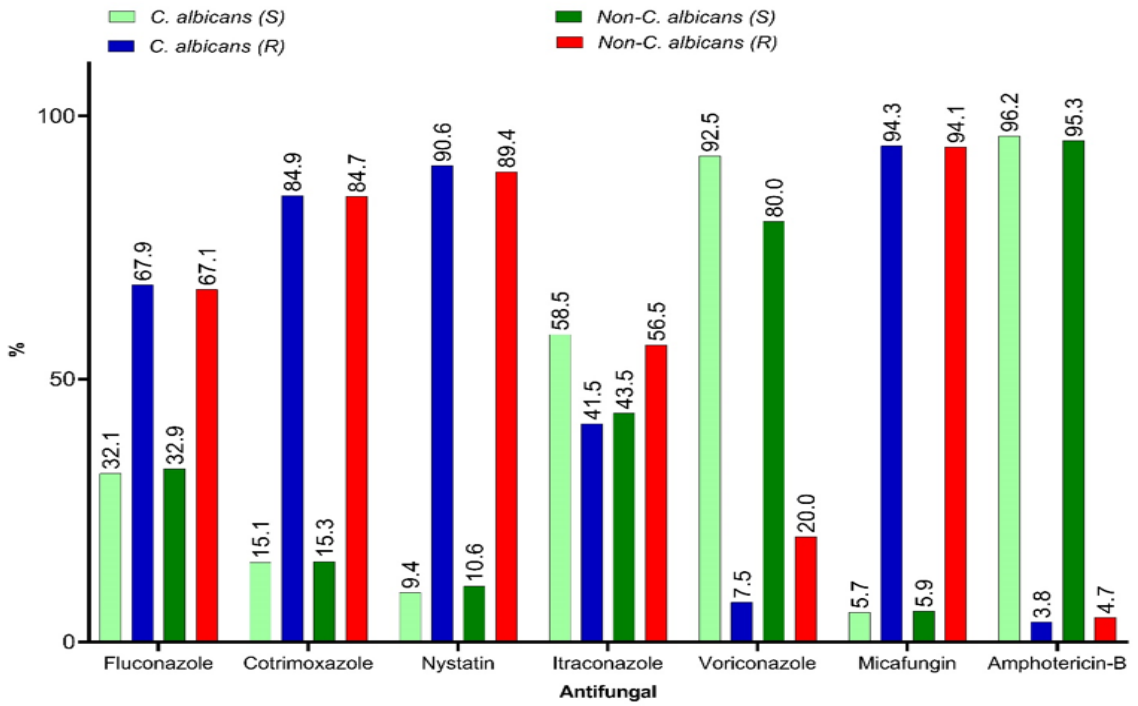



**Figure No.9 : Antifungal susceptibility of Candida and non-candida isolates.**

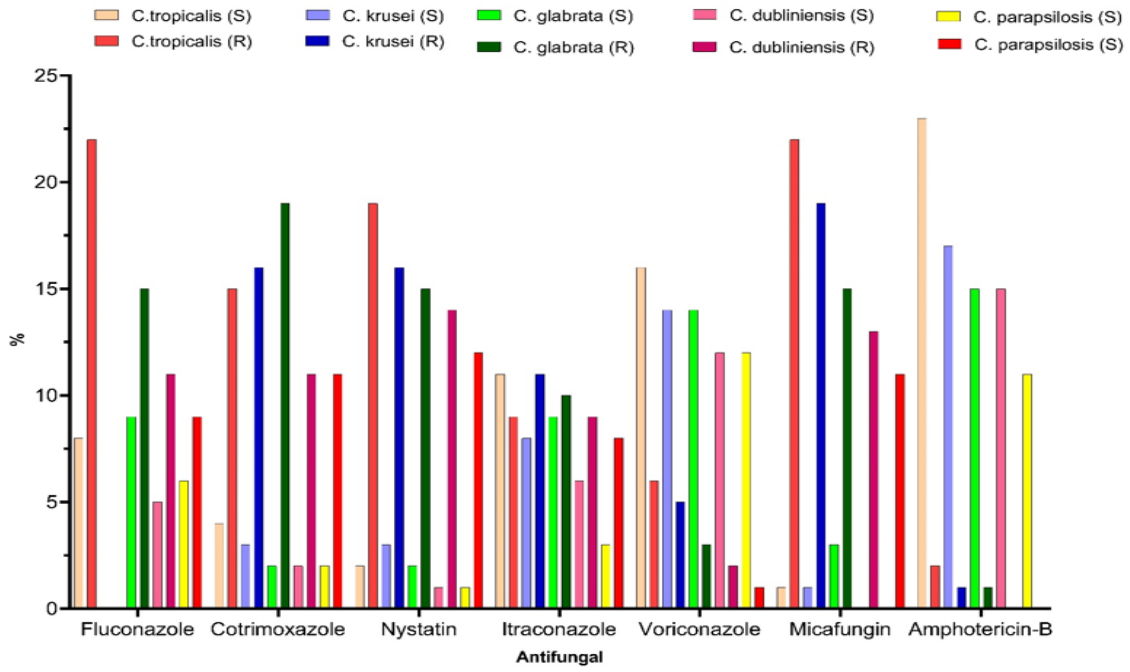



**Figure No. 10: Distribution of Antifungal susceptibility of non-candida isolates.**

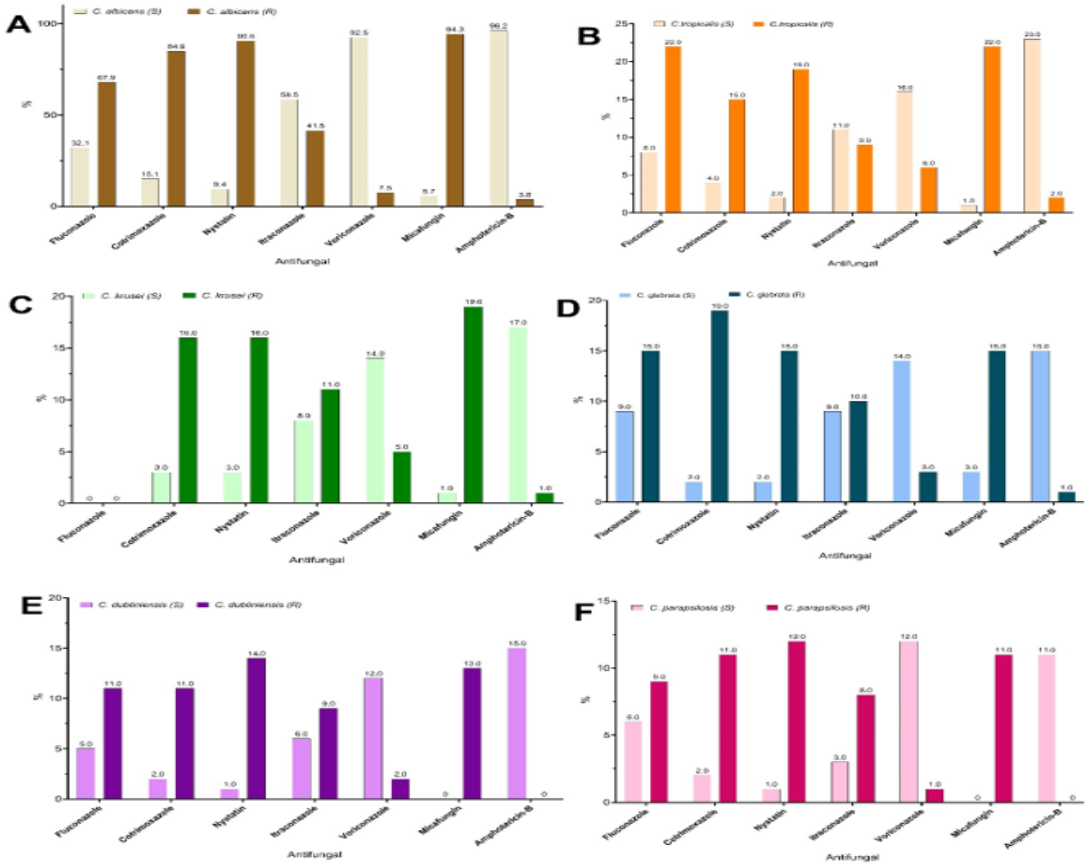



**Figure No. 11: Distribution of antifungal susceptibility of Candida and non candida isolates.**

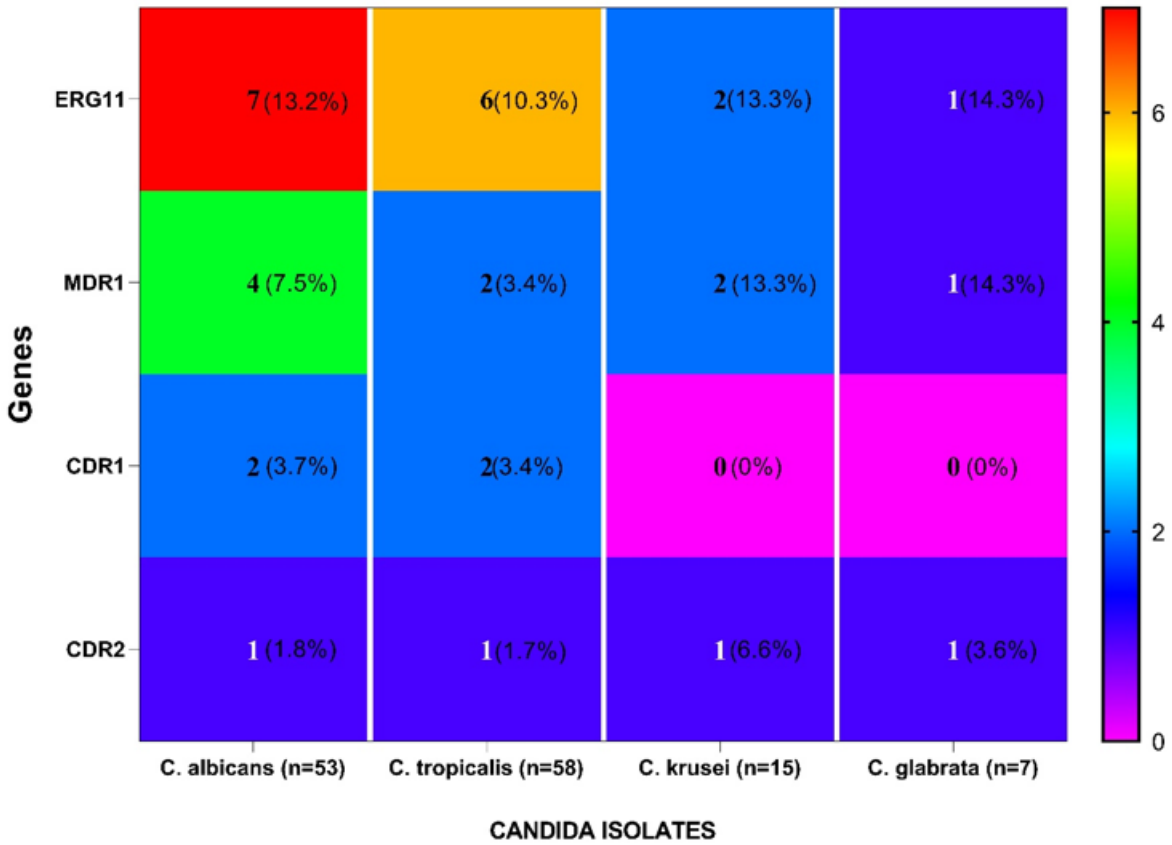



**Figure No. 12: Gene distribution of *Candida* and non-*Candida* species: ERG11, MDR1, CDR1, CDR2 AND ACTIN**

**Table No. 13: Gene distribution of *Candida* and non-*Candida* species: ERG11, MDR1, CDR1, CDR2**

| SPECIES / GENE | *Candida albicans* N=53 | *Candida tropicalis* N=58 | *Candida krusei* N=15 | *Candida glabrata*N=7 | Total gene |
|---|---|---|---|---|---|
| ERG11 | 7(13.20%) | 6(10.34%) | 2(13.33%) | 1(14.28%) | 16(11.59%) |
| MDR1 | 4(7.54%) | 2(3.44%) | 2(13.33%) | 1(14.28) | 9(6.52%) |
| CDR1 | 2(3.77%) | 2(3.44%) | 0 | 0 | 4(3.62%) |
| CDR2 | 1(1.88%) | 1(1.72%) | 1(6.66%) | 1(14.28%) | 4(3.62%) |

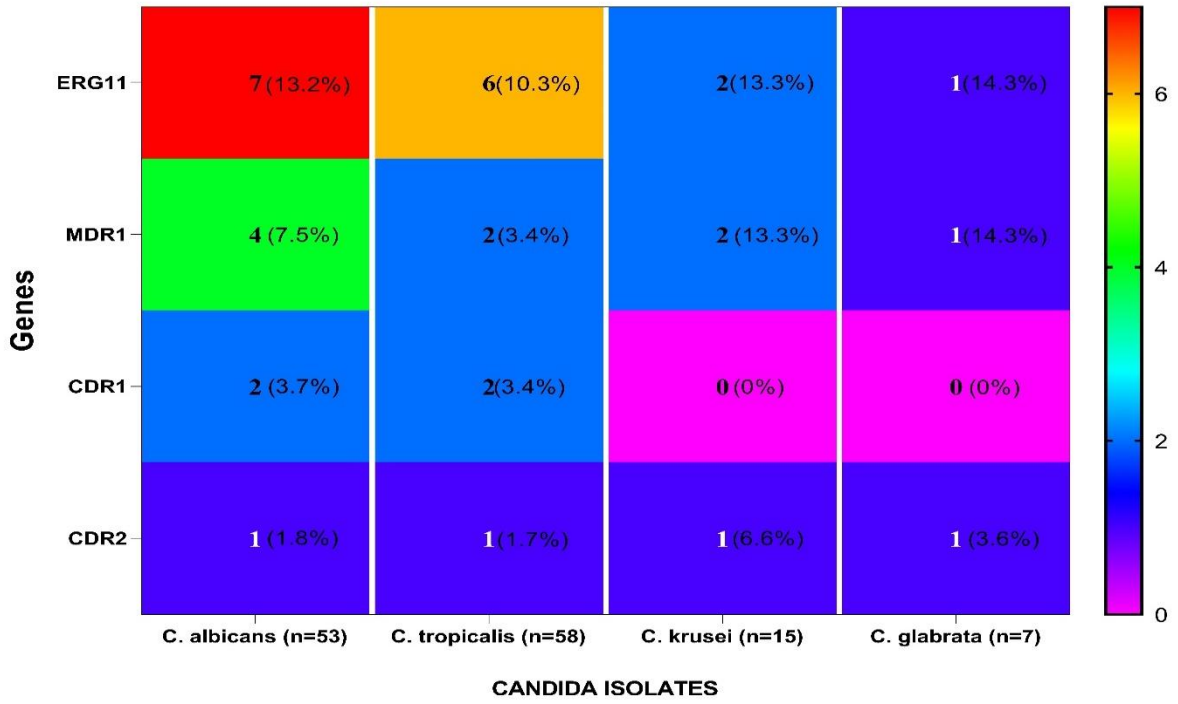
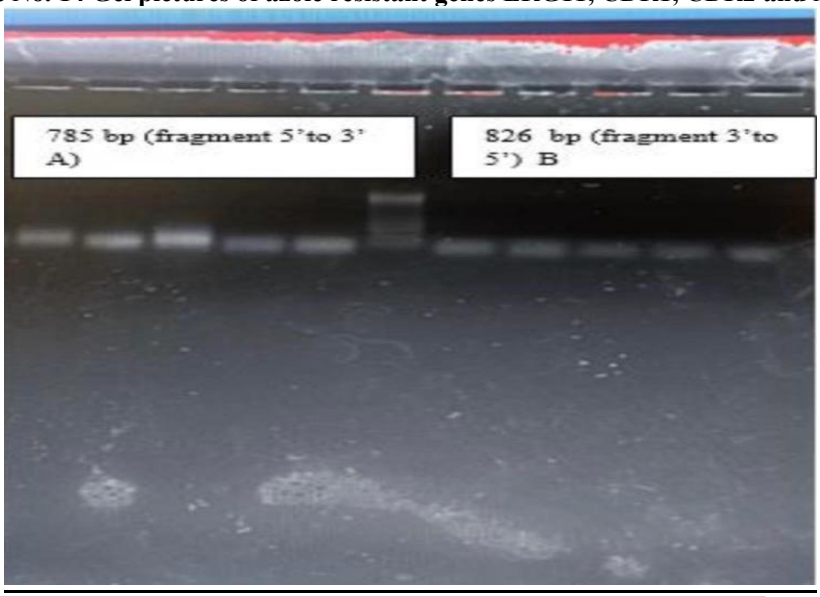


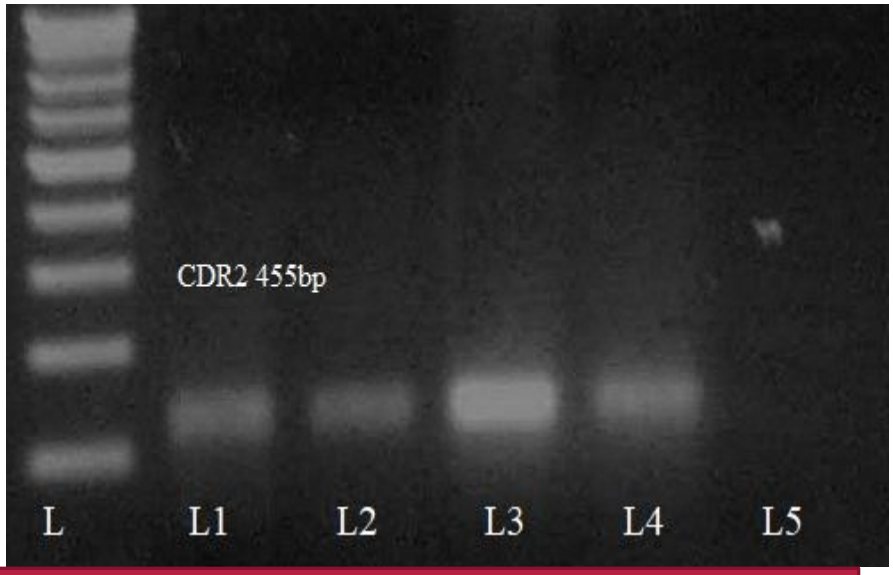

**Figure No. 13: Gene distribution of *Candida* and non-*Candida* species: ERG11, MDR1, CDR1, CDR2**

**Figure No. 14 Gel pictures of azole resistant genes ERG11, CDR1, CDR2 and MDR1**




Amplified erg11 gene in antifungal resistant *candida species* with positive control ATCC 10231. Lane L1,L2,L3,L4,L7,L8,L9,L10 corresponds to the positive control , L6 corresponds to the DNA ladder.


**The Gene Extraction CDR1 geneL is the Ladder, L1 corresponds to the Positive Control ATCC CDR1 resistant; L2 corresponds to the Negative control for CDR1; L3- L7 are the sample positive for**




The Gene Exctraction CDR2 gene:
L corresponds to the DNA Ladder; L1- L2 are the sample positive for CDR2 gene; L3 corresponds to the Positive Control for CDR2 gene; L4 corresponds to the sample positive for CDR2 gene; L5 is the Negative Control gene for CDR2 gene


**The Gene Extraction MDR1 gene**
**L1 is the sample positive for MDR1 gene, L corresponds to the DNA Ladder, L1-L2 is the Positive Control MDR1 resistant; L3, L6 are the sample positive for resistant gene MDR1, L7 negative control**

## 4. DISCUSSION

Targeting different resistance genes and a large sample size of highly resistant strains allows a better determination of the relevance of the different mutations, and to differentiate between causal mutations and polymorphisms.

The pharmacological target of azoles is the enzyme 14-α-demethylase (encoded by *ERG11*), an important enzyme in ergosterol biosynthesis. Acquired resistance to azoles may be caused by several mechanisms [16]. Mutations of the pharmacological target are able to change the enzyme's structure and may result in reduced binding affinity of the azoles to Erg11p [17]. Frequently, efflux pumps reduce the intracellular accumulation of azoles. The increased efflux is based on overexpression of *CDR1*/*CDR2* (Candida Drug Resistance) and *MDR1* (Multi Drug Resistance). Gain-of-function mutations in the transcription factors *TAC1* and *CgPDR1* can lead to higher gene expression of drug efflux pumps [10]

The purpose of the molecular characterization of *ERG11*, MDR1, CDR1 and CDR2 gene in the present study was to understand the molecular mechanism of azole resistant in *Candida* species isolated from various clinical samples.

According to Sony Paul et al and Sardari et al, the *ERG11* gene of fluconazole-resistant *C.albicans* was amplified, all the resistant isolates showed mutations in *ERG11 gene,* in particular, two sequences exhibited extensive mutations [38,41].

Many similar investigations addressing *ERG11* gene mutation reported different types of point mutations. However, these studies showed only a limited number of mutations, especially missense mutations.

In an Iranian study, researchers examined the up-regulation of CDR1, CDR2, MDR1 and ERG11 genes in two groups of *C.albicans* isolates. One group included isolates that were sensitive to treatment, while the other group consisted of resistant isolates. The findings of the study demonstrated that among these genes, MDR1 exhibited the most frequent occurrence of overexpression, followed by CDR1.

In the present study it was observed that, Prevalence of ERG 11 gene was 7(13.20%) followed by MDR1 9(6.52%) CDR1 4(3.62%) and CDR2 4(3.62%) respectively. which was quite similar with the studies done by Sardari et al, Ikenyi et al and Dovo et al, the prevalence in these studies were 4%, 3.17%, 9.79% respectively.[41,33,10]

**Table No. 14: Comparison of Prevalence of ERG 11 Gene among various studies.**

| Gene | Species | Present Study (2024) | Papon et al. (2013)[43] | Ben-Ami et al. (2012)[44] | Sardari A et al. 2019[41] | Ikenyi et al (2020)[33] | Dovo et al (2022)[10] |
|---|---|---|---|---|---|---|---|
| **ERG11** | *C. albicans* | 13.20% | 14.0% | 12.0% | 4% | 3.17% | 9.79% |
| | *C. tropicalis* | 10.34% | - | 9.5% | - | | |
| | *C. glabrata* | 14.28% | 13.0% | - | - | | |
| | *C. krusei* | 13.33% | - | - | - | | |

**Table No. 15: Comparison of Prevalence of CDR1 Gene among various studies.**

| Gene | Species | Present Study (2024) | Papon et al. (2013)[43] | Ben-Ami et al. (2012)[44] | Coste et al. (2007)[45] |
|---|---|---|---|---|---|
| **CDR1** | *C. albicans* | 3.77% | 8.0% | - | 6.0% |
| | *C. tropicalis* | 3.44% | - | - | - |
| | *C. glabrata* | 0% | - | - | - |
| | *C. krusei* | 0% | - | - | - |

**Table No. 16: Comparison of Prevalence of CDR2 Gene among various studies.**

| Gene | Species | Present Study (2024) | Coste et al. (2007)[45] | Khosravi Rad K et al (2017)[36] |
|---|---|---|---|---|
| **CDR2** | *C. albicans* | 1.88% | 2.0% | 87.5% |
| | *C. tropicalis* | 1.72% | - | |
| | *C. glabrata* | 14.28% | - | |
| | *C. krusei* | 6.66% | - | |

**Table No. 17: Comparison of MDR1 Gene among various studies.**

| Gene | Species | Present Study (2024) | Papon et al. (2013)[43] | Ben-Ami et al. (2012)[44] | Khosravi Rad K et al (2017)[36] |
|---|---|---|---|---|---|
| **MDR1** | *C. albicans* | 7.54% | 15.0% | 18.0% | 80% |
| | *C. tropicalis* | 3.44% | - | 5.0% | - |
| | *C. glabrata* | 13.28% | 12.0% | - | - |
| | *C. krusei* | 13.33% | - | - | - |

The widespread use of azole antifungal drugs has led to the development of drug-resistant isolates. Several molecular mechanisms that contribute to drug resistance have been identified, including increased mRNA levels for two types of

efflux pump genes: the ATP binding cassette transporter CDRs (*CDR1* and *CDR2*) and the major facilitator *MDR1*.

*MDR1*, *CDR1*, and *CDR2* are expressed early during logarithmic growth, *CDR4* is expressed late during logarithmic growth, and *CDR1* is preferentially expressed in stationary-phase cells. There is a small decrease in expression of these genes when the cells are grown in carbon sources other than glucose. While increased mRNA levels of efflux pump genes are commonly associated with azole resistance, the causes of increased mRNA levels have not yet been resolved. Southern blot analysis demonstrates that the increased mRNA levels in these isolates are not the result of gene amplification. Nuclear run-on assays show that *MDR1* and *CDR* mRNAs are transcriptionally overexpressed in the resistant isolate, suggesting that the antifungal drug resistance in this series is associated with the promoter and *trans*-acting factors of the *CDR1*, *CDR2*, and *MDR1* genes

Elisabete Ricardo et al in 2009 stated that Gene expression was quantified by real-time PCR, with and without ibuprofen, regarding CDR1, CDR2, MDR1, encoding for efflux pumps, and ERG11, encoding for azole target protein. A correlation between susceptibility phenotype and resistance gene expression profiles was determined. Ibuprofen and FK506 showed a clear synergistic effect when combined with fluconazole. Resistant isolates reverting to susceptible after incubation with ibuprofen showed CDR1 and CDR2 overexpression especially of the latter. Conversely, strains that did not revert displayed a remarkable increase in ERG11 expression along with CDR genes.

## 5. CONCLUSION

The emergence of resistant strains, including those becoming resistant to multiple drugs, has been increasingly reported in recent years. In addition, it has been demonstrated that such resistant phenotypes can develop over the course of an infection, and in response to treatment, which adds yet another threat to patients Still, we are far from having a broad understanding of how resistance towards antifungal drugs emerges in the context of infection or commensalism in yeast pathogens. Fortunately, recent developments in sequencing technologies are enabling us to catalog and trace the origins of mutations conferring resistance to antifungal drugs in different species.

The present study revealed the presence of four gene of *candida* species which is responsible for azole resistant, In this study it was observed that, prevalence of ERG 11 gene was 7(13.20%), CDR1 4(3.62%), CDR2 4(3.62%), and MDR1 9(6.52%) respectively.

In azole resistant, ERG11 expression was more predominant than azole resistive isolates in *candidaalbicans* followed by MDR1, CDR1 and CDR2 gene. For the past 20 years, many genes and mutation which promote fluconazole resistant's in clinical isolates especially in *candida albicans* has been revealed.

The high expression levels of ERG11, MDR-1, CDR1 and CDR2 genes in *C. albicans* isolates in various clinical samples highlights the important role of these genes in developing fluconazole resistance, causing treatment attempts to fail and leading to chronic infections.

Detecting the mechanism of resistance to antifungal compounds and identifying the *Candida* isolates in clinical laboratories are of high importance. Furthermore, the high prevalence of fluconazole-resistant strains of C.albicans and non-candida albicans in this particular geographical area in clinical samples indicate the presence of ERG11 gene.

**DECLARATIONS:**
**Conflicts of interest: There is no any conflict of interest associated with this study**
**Consent to participate:** There is consent to participate.
**Consent for publication:** There is consent for the publication of this paper.
**Authors' contributions: Author equally contributed the work.**

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