Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 8
Open Access

To Study The Molecular Characterization Of Mdr1 Gene And Cdr1 Gene With Association To Its Antifungal Resistance In Pathogenic *Candida* Species

Parveen Anjum Sheiq , Khutija Sarah*, Sumaira Qayoom, Dr. (WgCmdr) Md. Muzammil

Assistant Professor[1], Department of Basic Medical Sciences, Unaiza College of Medicine, Al Qassim University, KSA.
Assistant Professor [2]*, Department of Microbiology, Rama Medical College Hospital and Research Centre, Kanpur, Uttar Pradesh, India.
Assistant Professor[3] , Department of Microbiology, Sri Siddhartha Institute of Medical Sciences and Research Centre, Karnataka, India.
Assistant Professor[4], Department of Anesthesiology, Rama Medical College Hospital and Research Centre, Kanpur, Uttar Pradesh, India.

Corresponding Author: Dr. Khutija Sarah*
Email ID: dr.muzu@gmail.com

Cite this paper as: Parveen Anjum Sheiq , Khutija Sarah, Sumaira Qayoom, Dr. (WgCmdr) Md. Muzammil (2024). To Study The Molecular Characterization Of Mdr1 Gene And Cdr1 Gene With Association To Its Antifungal Resistance In Pathogenic Candida Species. *Frontiers in Health Informatics*, *13 (8) 6637-6653*

Abstract:

INTRODUCTION: Candidiasis is a highly pervasive infection posing major health risks, especially for immunocompromised populations. Its prevalence accounts for the most common type of opportunistic fungal infection affecting human heath globally, with more than a billion cases on a yearly basis. Pathogenic *Candida* species have evolved intrinsic and acquired resistance to a variety of antifungal medications.

AIM AND OBJECTIVE: To study the molecular characterization of MDR1 gene and CDR1 gene with association to its antifungal resistance in pathogenic *candida* species

MATERIAL AND METHODS: This was a cross sectional study conducted in the Department of Microbiology at a tertiary care centre. A total of 962 samples were screened. The Culture identification, specification, Antifungal Susceptibility testing was performed according to the CLSI guidelines. The DNA was extracted using the Qiagen DNA extraction kit and the resistant gene MDR1 and CDR1 gene was detected using the PCR.

RESULTS: In the present study out of 962 isolates, 51.1% (492) were culture positive, among them 28% (138) were Candida isolates. Out of which 53(38.4%) were *Candida albican*s while 85(61.6%) were Non-Candida albicans. Among Non-Candida albicans ,the frequency of *Candida tropicalis* was observed to be maximum with urine samples and least for ET secreation. It was observed that 112(81.1%) Candida isolates shows biofilm production ,While Phospholipidase enzymes production were observed in only 22(15.9%) of Candida isolates. It was observed that the maximum sensitivity was observed in Amphotericin-B (95%), followed by Voriconazole(85%) and itraconazole (49.2%). It was observed that the prevalence of associated genes in *Candida* with CDR1 with 3.8% and MDR1 with 5.7% was expressed.

CONCLUSION: The pathophysiology and clinical consequences of candidiasis can be better understood by characterising the virulence factors in Candida species that have been isolated from a variety of clinical specimens. To solve the changing problems caused by Candida infections and enhance patient outcomes in clinical practice, more research is necessary. A more thorough evaluation of azole resistance is made possible by taking into account the interaction of genetic alterations, phenotypic traits, and patient-related variables.
KEYWORDS: Antifungal susceptibility testing, Virulence factors, Fluconazole, DNA, PCR, MDR1, CDR1

INTRODUCTION

The Candida species are the main leading cause of fungal infections that are usually associated with wide range of infections either mild mucocutaneous candidiasis or severe invasive candidiasis with high morbidity and mortality rate [1] . Fungal pathogens known as Candida species are opportunistic and can cause a wide range of illnesses, from systemic infections to superficial mucocutaneous disorders. Eight to ten percent of the patients in a US research had blood samples tested positive for Candida [2,3].

The Conditions which make *C. albicans* pathogenic is its phenotypic switching ability, it can switch between different but stable phenotypic states, the way it retains the ability to mate but loses the ability to go through meiosis to complete sexual cycle. *C. albicans* is imperfect yeast that is capable to transitioning to hyphal form led by environmental factors. This results in changes in colonial morphology [4].

Candida species are commonly found as part of the normal human microbiome, but they can cause infections in certain situations. The isolation of Candida species is important in order to identify the specific species present, and to determine whether it is the cause of an infection. In invasive candidiasis, which is a serious infection that can affect the bloodstream, urinary tract, and other organs [5]. Isolation of Candida species from clinical specimens can help confirm the diagnosis of these infections, and can also be used to monitor the emergence of drug-resistant strains of Candida .

Even while *Candida albicans* is usually blamed for infections, there has been a noticeable trend in recent years towards treatment-resistant non-albicans Candida (NAC) species [6]. The fact that many NAC species show differing degrees of innate, acquired, or both resistance to widely used antifungal medications exacerbates the problem of NAC spp. emergence.

Azoles are a class of antifungal drugs that are commonly used to treat Candida infections. They work by inhibiting the growth of the fungus by binding to a specific enzyme, called cytochrome P450 14 alpha-demethylase, which is necessary for the fungal cell to grow and reproduce. Azoles include drugs such as fluconazole, itraconazole, and voriconazole. Fluconazole is a member of the azole class of antifungal drugs and is commonly used to treat Candida infections [7,8]. It is effective against a wide range of Candida species, including C. *albicans*, C. *glabrata*, C. *tropicalis*, C. *krusei*, and C. *parapsilosis.*

Various antifungals are used for treatment of *Candida* infections, among them, azoles which showed good activity and are relatively safe, however, resistance to this group is occurring more frequently [7], where antifungal resistance is a major concern in clinical practice and becoming a major problem. Intensive and long-term use of antifungal drugs lead to decline in susceptibility and resistance patterns of Candida species [8-10].

Recently, resistance to common antifungals has been reported in different *Candida* species[11,12]. Fluconazole antifungal works by inhibiting the growth of *Candida albicans* by targeting the fungal cell membrane. The drug targets an enzyme called 14-alpha-demethylase, which is responsible for converting lanosterol to ergosterol and is a component of the fungal cell membrane [13]. Without this enzyme, the fungal cell membrane can't be produced and the fungus can't grow. This leads to the death of the fungus, effectively treating the infection.

Fluconazole is available in both oral and intravenous forms and can be used to treat both acute and chronic *Candida albicans* infections. The oral form is commonly used to treat thrush and vaginal yeast infections, while the intravenous form is used to treat more serious and invasive infections.

However, the prevalence of drug resistance to fluconazole among *Candida albicans* is an increasing concern in the medical community. This means that in some cases, the infection may not respond to treatment with fluconazole, leading to treatment failure. To overcome this problem, combination therapy using different classes of antifungal medications may be effective in treating fluconazole-resistant *Candida albicans* infections [15].

In the last 5 years, through several molecular mechanism *C. albicans* have developed huge resistance to the antifungal drugs [16]. ERG11 is a crucial gene found in the yeast Saccharomyces cerevisiae and its null mutant which can only grow in anaerobic conditions when supplemented with ergosterol. However, in the fungus *Candida albicans*, the null mutant of the gene can grow slowly under aerobic conditions even without ergosterol. This gives rise to the accumulation of toxic sterol intermediates and the emergence of spontaneous suppressor mutations that inactivate C-5 sterol desaturase, which is encoded by the ERG3 gene. Ergosterol biosynthesis is a primary target of several antifungal drugs, including azoles such as fluconazole and ketoconazole.

One of the known potential resistance mechanism of azoles is alteration in the ergosterol syntheses pathway. *Candida* species can develop resistance by mutation/s in the gene *(ERG11)* which codes for the enzyme 14α-demethylase [17].

ERG11-2 is a gene that plays a crucial role in the development of resistance to fluconazole. This resistance is caused by mutations in the gene that lead to an increase in its expression. Azoles, such as fluconazole, target the 14-α-steroldemethylase enzyme, which is encoded by the ERG11 gene. This enzyme is responsible for the biosynthesis of ergosterol [13, 18].

MDR1 is overexpressed in many fluconazole-resistant strains of *Candida albicans*. This overexpression is a major cause of resistance to fluconazole and other antifungal agents. The CDR1 gene is one of the main genes associated with drug resistance in Candida albicans. Its often associated with energy-dependent drug efflux in clinical isolates that are resistant to fluconazole. Overexpression of plasma membrane efflux pumps. Two types of azole transporters in *C. albicans* have been identified: the major superfamily transporter encoded by MDR1 and the ATP-binding cassette (ABC) transporters encoded by CDR1 and CDR2. These pumps differ in the specificity of the azole molecule and in the source of energy used to translocate the compounds across the cell membrane. The Cdr proteins are primary transporters able to transport all azole compounds using the hydrolysis of ATP; on the contrary, Mdr1p pump is a secondary transporter which uses proton gradient for extrusion of fluconazole [19,20]. Consequently, upregulation of MDR1 is responsible for fluconazole resistance and upregulation of ABC
transporters results in multi azole resistance [21] .

The effect of overexpression of these efflux pumps is the decreased intracellular concentration of azole available for inhibition of the target enzyme (lanosterol 14α- demethylase) . Mutations in the transcription factors TAC1 (transcriptional activator of CDR genes) and MRR1 (multidrug resistance regulator 1) are responsible for upregulation of CDR1/CDR2 and MDR1, respectively To date, nineteen point mutations in different domains of TAC1 have been identified and fifteen mutations for MRR1 [22].

There are several methods for identifying Candida species including; Microscopic examination by observing its morphological features, Biochemical tests by looking at its ability to ferment specific sugars, produce specific enzymes or utilize certain substrates, Molecular methods such as PCR and DNA sequencing and advanced technology such as MALDI-TOF MS which is a rapid, accurate and cost-effective method for identification of microorganisms.

However, overuse of this drug could lead to the emergence of resistance, which means that the fungus could become

resistant to the drug and be harder to treat. Therefore, it's important to use fluconazole only when necessary and to use other treatments when possible [22].

Given these concerns, it is essential to identify the extent of the problem and determine the species of Candida causing infections, as well as their susceptibility to antifungal agents. Additionally, detecting the MDR1 and CDR1 gene in fluconazole-resistant *Candida albicans* can help to better understand the mechanisms of resistance and aid in the development of more effective treatments.

Therefore, the present study was undertaken for the detection of the molecular characterization of MDR1 gene and CDR1 gene with association to its antifungal resistance in pathogenic *candida* species

MATERIAL AND METHODS

This was a Cross sectional study carried out in the Department of Microbiology at a tertiary care centre, for a period of 1 year i.e, November 2023 to November 2024. The Demographic details and clinical history along with the relevant clinical investigations was recorded after the informed consent. Candida isolates from all clinical specimen in pure culture were included in the study whereas, repeat isolates from same clinical specimen of same patient and isolation of Candida species from mix culture were excluded from the study.

LABORATORY EXAMINATION

Isolation and Identification of *Candida albicans*

Samples- Urine, sputum, blood, vaginal swabs collected using aseptic precautions as per the Standard CLSI guidelines.

MYCOLOGICAL EXAMINATION : Specimens such as mucosal scrapings were collected under aseptic precautions and examined microscopically in 10% potassium hydroxide (KOH) solution for the presence of fungal elements. Whenever the scraping was positive for budding yeast cells, hyphae or pseudohyphae, inoculation was done on Sabouraud's Dextrose Agar (SDA) (Media of pH 6.5) with chloramphenicol (0.05mg/ml). Duplicate slants were maintained for all specimen. All the inoculated slants were duly numbered and incubated at 37o C for a period of 24-48 hours, with everyday observation. Candida colonies appeared as white or cream coloured, smooth with a yeasty odour.

Culture- Samples collected was inoculated on to Blood agar, Macconkey agar and two tubes of Sabourauds Dextrose agar, one tube incubated at 25◦c and other at 37◦c for 24 to 48 hrs. Growth of Candida was identified by colonial characteristics as white to creamy and pasty colonies and Gram staining reveal Gram positive budding yeast cells . Then the speciation to *C.albicans* was done by standard Conventional techniques

LABORATORY DIAGNOSIS

Direct Examination Clinical specimens from diseased skin or nails can be collected by scraping the affected area or by the use of swabs. Preferred method for direct examination of clinical specimens from cutaneous and nail candidiasis is the wet mount technique. Specimens have to be treated in most cases, with a keratinolytic generally 10-30% KOH, which facilitates microscopic examination of the specimen.

Sample collection: A total of 962 different samples were collected aseptically which include Blood, urine, sputum, Pus, ET secretions, Throat swab,vaginal swab, pleural fluid, ascitic fluid. These samples were immediately transferred to the microbiology department for further processing.

Sample Processing: Direct gram staining were performed to see the presence of yeast and pseudohyphae of Candida species from the different samples. Urine samples were inoculated on CLED agar while others were inoculated on Blood agar for 24 hrs at 37ºC. Then the colonies from these plates were cultured on SDA and CHROMagar and incubated for 24-48hrs at room temperature.

Species Identification: Candida species were identified phnotypically by Gram staining, Germ tube test, Colonies on

CHROMagar, Biochemical tests like urease test & Carbohydrate assimilation test.

Antifungal susceptibility testing: A total of 138 isolates of Candida species from different clincal specimens like blood,BAL,Urine, Pus ,Et secreation and Vaginal secreation were included in our study. Antifungal sensitivity of Candida isolates was done by Kirby-Bauer disc diffusion method. Mueller Hinton agar supplemented with 0.2% glucose and 0.5µg/ml methylene blue dye medium (MH-GMB) was used for this purpose against azole group Fluconazole 25ug from Hi-media Laboratories Pvt Ltd India. The broth micro dilution method was done to determine the minimum inhibitory concentrations (MICs) according to the CLSI guidelines 2024 [23].

Molecular Identification of CDR1 gene of Fluconazole Resistant *Candida albicans*

The DNA was isolated using the Qiamp DNA Blood Mini Kit (QIAGEN, Germany) as per the manufactures guidelines. The DNA was eluted in 60 µl elution buffer and preserve at -20 °C till PCR analysis. For amplification of the target gene, PCR was carried out in a 50 µL reaction mixture with 35 no. of cycles. The primers were purchased from "**Saha gene'** and was reconstituted with sterile double distilled water based on the manufacturer's instruction.

Figure No.1: The Reagents used for the DNA Extraction

CDR1:

Target Gene	Primer sequence	Length (bp)	Reference
CDR1	CAATCACATTCGTCCTGGTTC TTGAAAGCCAAGGACATCAC	387bp	[24]

Table No. 1 : Primers used to amplify CDR1 gene fragment.

Polymerase Chain Reaction (PCR)

For the PCR amplification, 2 µl of template DNA was added to 18 µl reaction containing 10 µl of Qiagen master mix, 2 µl of primer mix (1 µl each of the respective forward and reverse primers) and 6 µl of molecular-grade water.

The cyclic conditions for CDR1 gene, initial denaturation at 95 °C for 15 min, 30 cycles of 94 °C for 30 s, 59 °C for 1 min 30 s and 72 °C for 1 min 30 s were followed by extension of 72 °C for 10 min.

The PCR cycling conditions

Step	Program CDR1 Time	Temperature	Cycles
Initial denaturation	15 min	95 ºC	
Denaturation	30 s	94 ºC	
Annealing	1 min30 s	59 ºC	30
Extension	1 min 30 s	72º C	
Final extension	10 min	72º C	

Table No. 2 : The PCR cycling conditions to amplify CDR1gene fragment.

MDR1:

Target Gene	Primer sequence	Length (bp)	Reference
MDR1	ATGTTGGCATTCACCCTTC GAAAACTTCTGGGAAAACTGG	426bp	[24]

Table No. 3 : Primers used to amplify MDR1 gene fragment.

Polymerase Chain Reaction (PCR)

For the PCR amplification, 2 µl of template DNA was added to 18 µl reaction containing 10 µl of Qiagen master mix, 2 µl of primer mix (1 µl each of the respective forward and reverse primers) and 6 µl of molecular-grade water.

The cyclic conditions for CDR1 gene, initial denaturation at 95 °C for 15 min, 30 cycles of 94 °C for 30 s, 59 °C for 1 min 30 s and 72 °C for 1 min 30 s were followed by extension of 72 °C for 10 min.

The PCR cycling conditions

Step	Program MDR1 Time	Temperature	Cycles
Initial denaturation	15 min	95 ºC	
Denaturation	30 s	94 ºC	
Annealing	1 min30 s	59 ºC	30
Extension	1 min 30 s	72º C	
Final extension	10 min	72º C	

Table No. 4 : The PCR cycling conditions to amplify MDR1gene fragment.

The Agarose gel preparation and visualized by Gel Doc™ EZ Gel Documentation System

- 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.
- **STATISTIC ANALYSIS**

Data along with statistic was recorded by the Microsoft Excel. The values were represented in Numbers percentage and bar diagram..

RESULTS

Out of 962 different samples, 51.1% (492) were culture positive, among them 28% (138) were Candida isolates. Out of which 53(38.4%) were *Candida albican*s while 85(61.6%) were Non-Candida albicans.

Among Non-Candida albicans ,the frequency of *Candida tropicalis* was observed to be 58 (68.2%). Maximum number of Non-Candida albicans were isolated from Urine samples (44.7%) followed by Vaginal swab (22.3%) and sputum(20%) While frequency of *C.albicans* was found more in Sputum sample (39.6%), followed by urine (24.5%),Vaginal swab (22.6%).

It was observed that 112(53.5%) Candida isolates shows biofilm production ,While Phospholipidase enzymes production were observed in only 21(10.04%) of Candida isolates. It was observed that the maximum sensitivity was observed in Amphotericin-B (95%), followed by Voriconazole(85%) and itraconazole (49.2%).

Out of 138 Candida isolates, 53 (38.4%) were *Candida albican*s while 85 (61.6%) were Non-Candida albicans. Among Non-Candida ablbicans,the frequency of *Candida tropicalis* was 58 (68.2%) followed by *Candida krusei*15 (17.6%),*Candida glabrata* 7 (8.23%) while 5 (5.8%) belongs to other group [Table no. 5].

Maximum number of Non-Candida albicans were isolated from Urine samples (44.7%) followed by Vaginal swab (22.3%), sputum(20%),ET secretions (7.05%), pus(7.07%), & Blood (1.17%). While pleural fluid and ascitic fluid showed no growth. While frequency of *C.albicans* was found more in Sputum sample(39.6%), followed by urine(24.5%),Vaginal swab (22.6%), pus (5.6%), blood (3.7%), and ET secretions(3.7%).

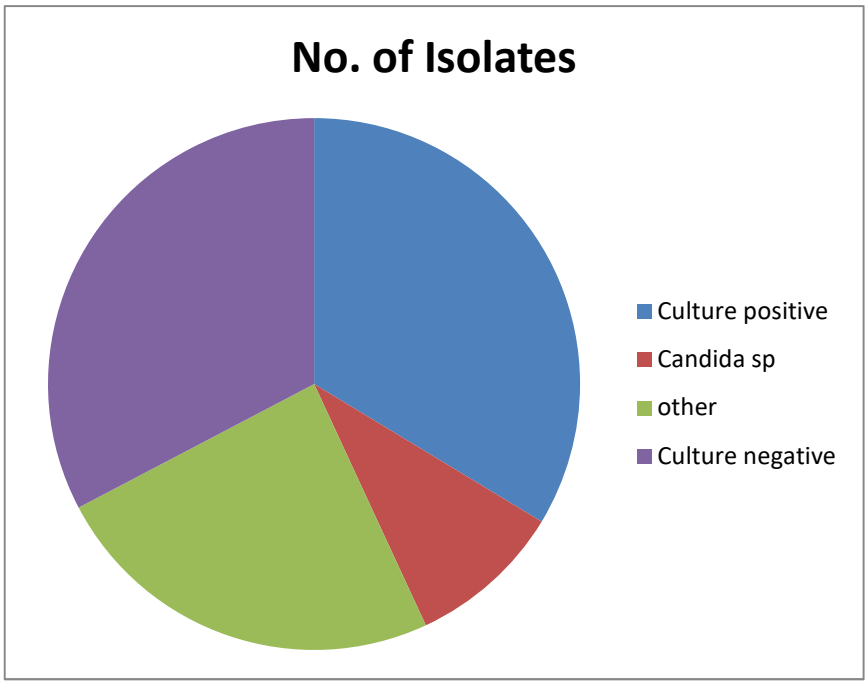
Out of these, 112(53.5%) Candida isolates shows biofilm production, among them *Candida troipcalis* shows maximum number of biofilm production (58.03%) followed by *C.albicans (37.5%)* and least were observed in *C.krusei* (2.67%) and *C.glabrata (1.78%)*. While Phospholipidase enzymes production were observed in only 21 (10.04%) of Candida isolates.

Antifungal susceptibility test shows maximum sensitivity towards Amphotericin-B (94.7%), Voriconazole(85.1%) & itraconazole (49.7%) while fluconazole (33%), cotrimoxazole (15.3%), nystin(10.4%), shows least sensitivity gainst Candida isolates as illustrated in table 5.

TOTAL SAMPLE	**FREQUENCY**	**PERCENTAGE**
Culture positive	492	51.1%
Candida sp	138	28%
other	354	71.9%
Culture negative	478	49.6%
Total	962	100%

Table No.5 : Frequency of culture positive and culture negative.

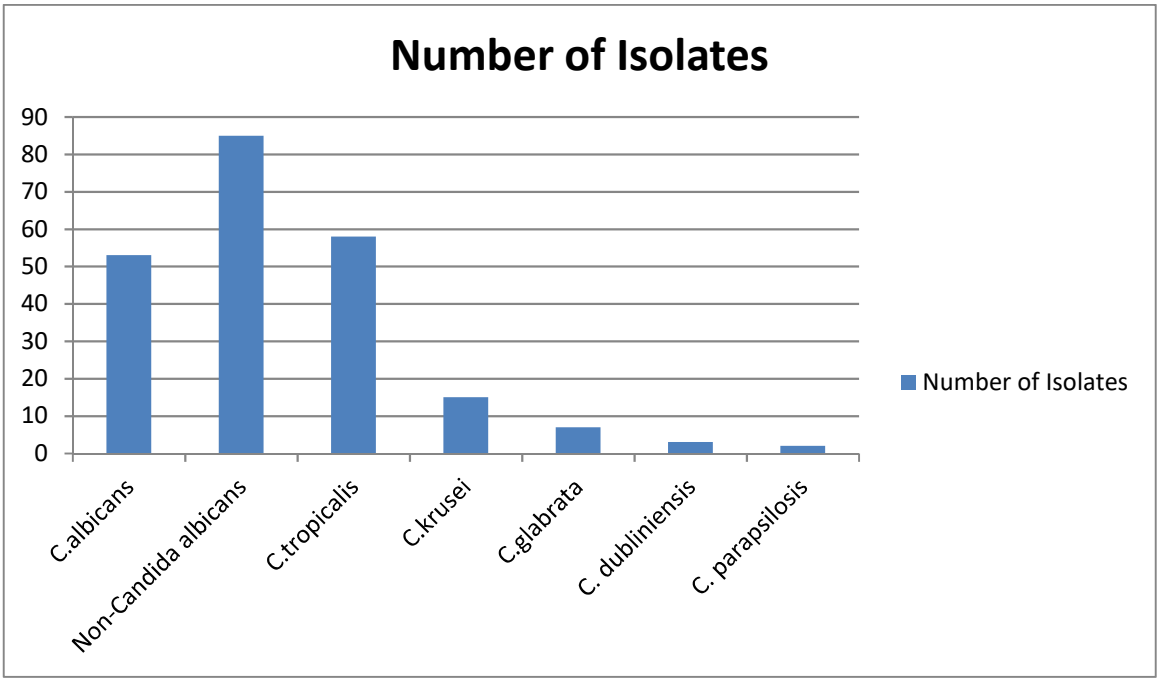
In this table 51.1% were culture positive out of which 28% were Candida isolates.



Graph No. 1: Graphical representation of of culture positive and culture negative

CANDIDA ISOLATES	No. of Isolates	PERCENTAGE
C.albicans	53	38.4%
Non-Candida albicans	85	61.6%
C.tropicalis	58	68.2%
C.krusei	15	17.6%
C.glabrata	7	8.23%
C. dubliniensis *C. parapsilosis*	3 2	3.5% 2.3%
TOTAL	138	100%

Table No. 6: Frequency of Candida isolates



Graph No. 2: Graphical representation of species distribution

From the Table no. 2 it was clear that out of 138 Candida isolates, 53 (38.4%) were *C.albicans* while 61.6% were Non-Candida albicans, among them frequency of *C.tropicalis*were more(68.2%) than other non-Candida albicans.

SAMPLE	*C.albicans* (n=53)	PERCENTAGE	Non-Candida albicans (n=85)	PERCENTAGE
Urine	13	24.5%	38	44.7%
Pus	3	5.6%	4	7.07%
Vaginal swab	12	22.6%	19	22.3%
Blood	2	3.7%	1	1.17%
Sputum	21	39.6%	17	20%
ET secretions	2	3.7%	6	7.05%
Pleural fluid	0	0%	0	0%

Ascitic fluid	0	0%	0	0%

Table No.7 : Frequency of *C.albicans*& Non-Candida albicans among different samples

In this table it was observed that maximum number of *C.albicans* were isolated from Sputum samples (39.6%) while more number of Non-Candida albicans were isolated from Urine sample (44.7%).

VIRULENCE FACTORS	FREQUENCY	PERCENTAGE
Biofilm production	112	81.1%
C.albians	42	30.4%
C.tropicalis	65	47.1%
C.glabrata	2	1.4%
C.krusei	3	2.1%
others	0	0
Phospholipidase enzyme	22	15.9%
C.albians	17	12.3%
C.tropicalis	3	2.1%
C.glabrata	2	1.4%
others	0	0.00%

Table No. 8: Frequency of virulence factors among Various Candida isolates.

In this table it was observed that Biofim production present in 81.1% of Candida isolates, among them mostly from *C.tropicalis*(47.1%), while production of Phospholipidase enzyme were observed only in 15.9% of isolates.

Table No. 9: Antifungal Drug Resistance Patterns .

ANTIFUNGAL DRUGS	NO. OF ISOLATES	SENSITIVITY(%)(n=138)
Fluconazole	45	33%
Cotrimoxazole	21	15.2%
Nystin	14	10.1%
Itraconazole	68	49.2%
Voriconazole	117	85%
Micafungin	8	5.7%
Amphotericin-B	132	95 %

Antifungal Drug	Sensitivity (%)	Resistance (%)
Fluconazole	33% (45)	67.3% (93)
Cotrimoxazole	15.2% (21)	84.7% (117)
Nystatin	10.1% (14)	89.8% (124)
Itraconazole	49.2% (68)	50.7% (70)
Voriconazole	85% (117)	15.2% (21)
Micafungin	5.7% (8)	94.2% (130)
Amphotericin-B	95.6% (132)	4.3% (6)

Table No. 10:Antifungal susceptibility of Candida isolates.

In this table,the maximum sensitivity was observed in Amphotericin-B(95%), followed by Voriconazole(85%) and itraconazole(49.2%).

Candida gene distribution, specifically for genes associated with resistance CDR1 and MDR1.

Species	**Total Samples**	**MDR1**	**CDR1**
Candida albicans	**53**	**3**	**2**
Candida tropicalis	**58**	**2**	**1**
Candida krusei	**15**	**2**	**0**
Candida glabrata	**7**	**1**	**1**
Other non-*Candida* spp.	**5**	**0**	**0**
Total	**138**	**8**	**4**

Table No. 11: Gene Distribution of *Candida* and Non-*Candida* Species: *ERG11*, *MDR*, *CDR1*, and *CDR2*.

Species	**Total Samples**	**MDR1 (5.7%)**	**CDR1 (3.8%)**
Candida albicans	53	3	2
Candida tropicalis	58	2	1
Candida krusei	15	2	0
Candida glabrata	7	1	1
Other non-*Candida* spp.	5	0	0
Total	138	8	4

Table No. 12 (A.): Gene Distribution of *Candida* and Non-*Candida* Species: *MDR*1 and *CDR1*

Species	**Total Samples**	**MDR1**	**CDR1**	**Resistant Genes Distribution**
Candida albicans	**53**	**3**	**2**	**resistant to MDR1 are 3 and 2 to CDR1**
Candida tropicalis	**58**	**2**	**1**	**2 resistant to MDR1 and 1 to CDR1**
Candida krusei	**15**	**2**	**0**	**2 resistant to MDR1 and 0 to CDR1**
Candida glabrata	**7**	**1**	**1**	**1 resistant to MDR1 and 1 to CDR1**

Other non-*Candida* spp.	**5**	**0**	**0**	-
Total	**138**	**8 (5.7%)**	**4 (3.8%)**	-

Table No. 12 (B.) : Gene Distribution of *Candida* and Non-*Candida* Species: *MDR*1 and *CDR1*

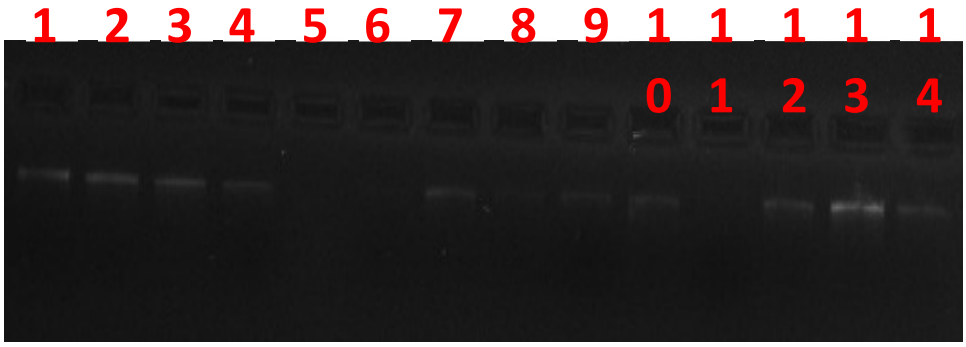


Figure No.2 : The DNA Extraction

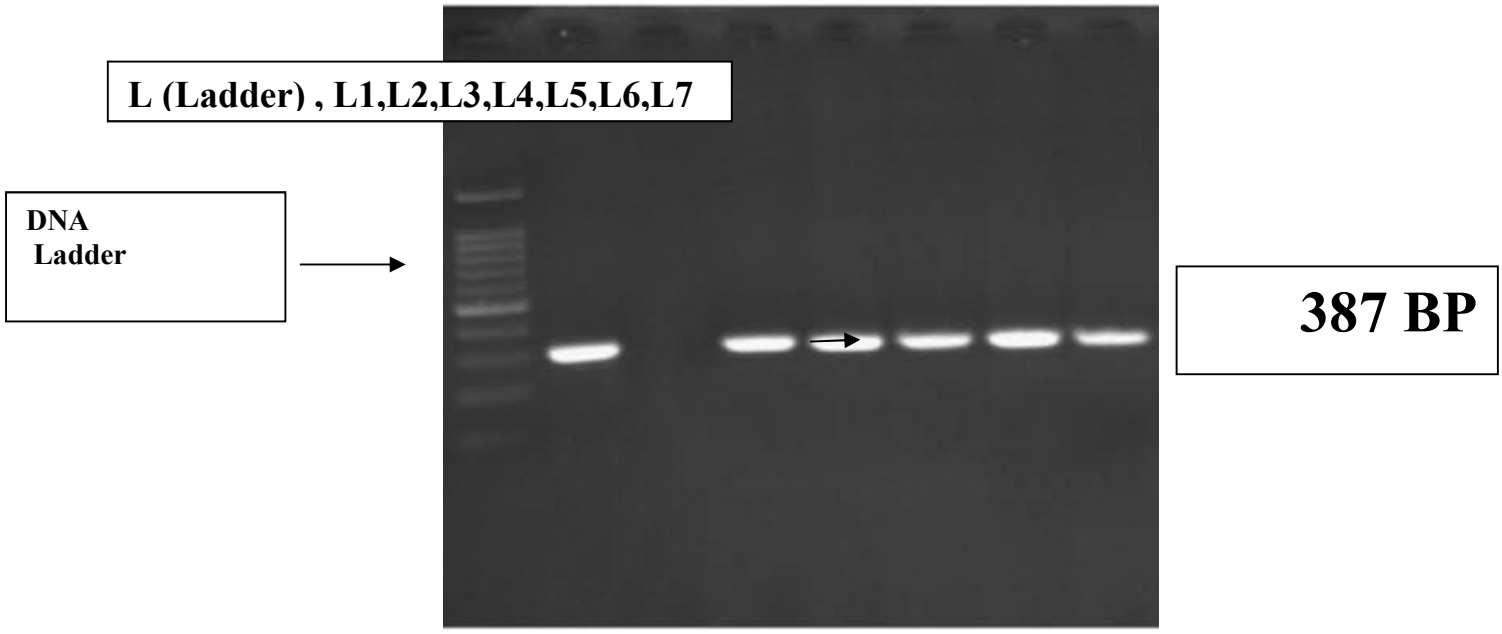


Figure No. 3: The Gene Extraction CDR1 gene
L is the Ladder, L1 corresponds to the Positive Control ATCC CDR1 resistant; L2 corresponds to the Negative control for CDR1; L3- L7 are the sample positive for resistant gene CDR1

L1,L2,L3,L4,L5

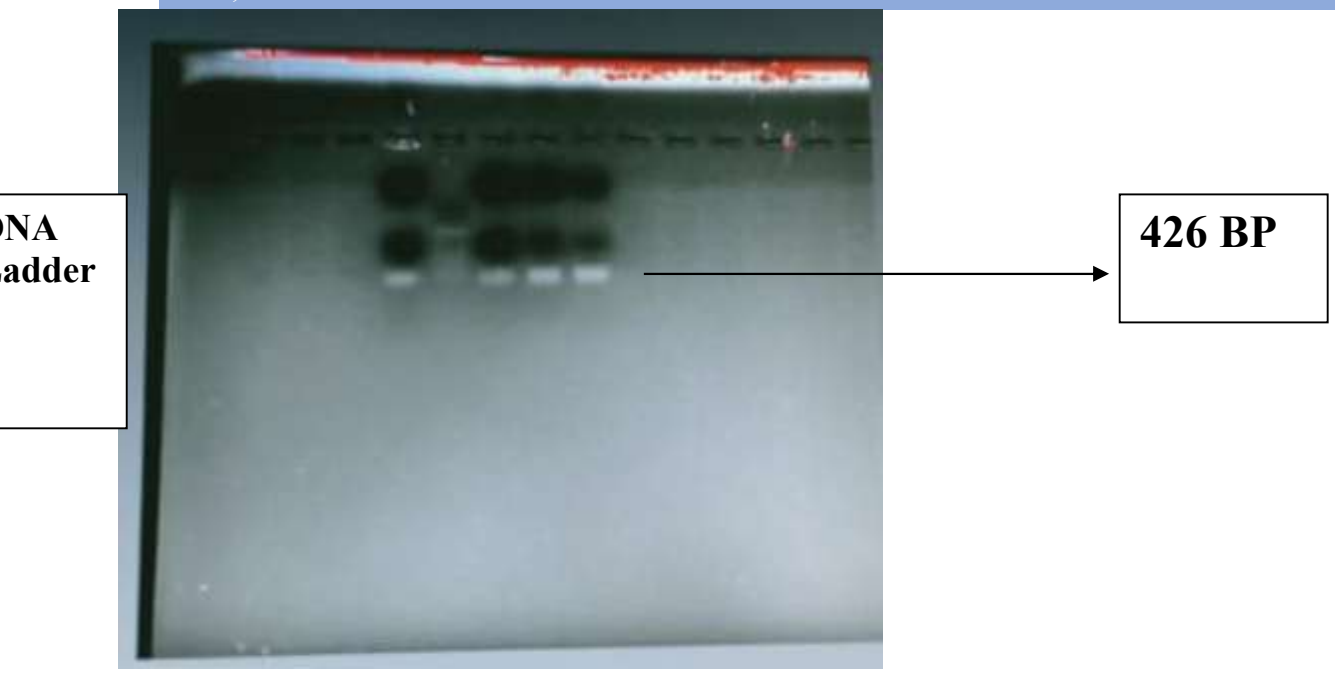


Figure No. 4: The Gene Extraction MDR1 gene
L1 is the sample positive for MDR1 gene, L2 corresponds to the DNA Ladder, L3 is the Positive Control ATCC MDR1 resistant; L3, L4 are the sample positive for resistant gene MDR1

Gene	CDR1	MDR1
Present study	4 (3.8%)	8 (5.7%)

Table No. 13: Distribution of *Candida* Genes

This table reflects the observed prevalence of associated genes in *Candida* in the present study with CDR1 with 3.8% and MDR1 with 5.7%.

DISCUSSION

Candida spp is a member of the normal flora of the skin, mucous membrane and gastrointestinal tract. They are endogenous opportunistswhich cause secondary infection in individuals with underlying immunocompromised conditions. Candidiasis is a common fungal disease in humans. An increase in the prevalence of non-albicans species has been noted during the last decades because of increasing use of azoles. This study aims to Spectate Candida using chromogenic medium [5].

Candida tropicalis, *C. krusei* , *C. glabrata*, and *C. parapsilosis* are the prominent causes of candidiasis following *C. albicans*. Distribution of these non-albicans species differs throughout the world mostly due to the medical state of patients, geographic distribution, age and gender [25].

In the present study out of 138 Candida isolates were obtained from different clinical specimens over a two years duration. Maximum *C. albicans* were isolated from sputum followed by urine, vaginal swab, pus, and blood representing 39.6%%, 24.5%, 22.6%%, 5.6%, and 3.7% respectively. Furthermore, a higher prevalence of non-albicans species was detected in urine (44.7%) followed by vaginal swab (22.3%), sputum (20%), ET secretions (7.05%), and pus (7.07%).

Our study revealed that the majority of Candida species were isolated from sputum, urine and vaginal swab which indicates a higher incidence of candida species induced urinary and respiratory tract infections. This study was in accordance to the study conducted by the other research investigator Vignesh Kanna B. et al where majority of isolates were from high vaginal swab (34%) followed by sputum (28%), urine (18%), pus from surgical sites and others constituted to 20%. *Candida albicans* (51%) was the most common *candida* species, followed by *C. tropicalis* (25%), *C. krusei* (16%), *C. glabrata* (6%) and *C. dubliniensis* (1%) [26]. There was another study by Sharma et.al [415] in 2023 where Urine 59.4%, Respiratory specimen (ET secretions and Broncho-alveolar lavage) 13.7%, Pus 5.2% and Blood 9.8% was recorded [27].

Potential clinical importance of species level identification has been recognized as Candidaspecies differ in the expression of virulence factors and antifungal susceptibility [28]. Candida species also have a direct impact on the choice of empirical antifungal therapy and clinical outcome. Non-albicans candida species are on the rise due to increasing immunocompromised condition.

Predominance of *C. albicans* was also seen in a study by Manjunath et al [29]. However,higher incidence of non-albicans candida ranging from 54-74% have been seen in numerous studies [30-32]. Among the non-albicans candida, *C. tropicalis* is reported to be the most predominant species, which was found in accoradance to the current study.

In the present study the prevalence of associated genes in *Candida* with CDR1 was observed to be 3.8% and MDR1 with 5.7%. This study was parallel to the study conducted by the other research investigator al. Coste et al., Ben-Ami et al. and Papon et al. where the expression of CDR1 was 6%, MDR was not expressed, CDR1 with not expressed whereas MDR1 gene with 18% and MDR1 gene with 15% , CDR1 gene with 3.7% respectively [33-35].

Resistance to azoles, particularly fluconazole, is rising worldwide. Different studies reported various profiles of *CDR1* and *MDR1* gene expression. Alterations in membrane permeability or changes in drug efflux pumps activities can contribute to azole resistance. Evaluating ergosterol levels, which are essential components of fungal cell membranes, can help assess the role of membrane-related modifications in resistance.

There is an interplay of different resistance mechanisms, including ones that were not investigated [36]. Overexpression of efflux pumps seems not to be the main azole resistance mechanism, as the EGR11 missense point mutation is the primary one, yet it contributes to the overall resistance. The expression of resistance genes in *Candida albicans* and *Candida tropicalis* is effective in increasing biofilm formation and the occurrence of candidiasis.

It is crucial to consider the interplay of genetic modifications, phenotypic characteristics, and patient-related factors, allowing for a more comprehensive assessment of azole resistance [37,38].

CONCLUSION

The study observed a notable correlations between antifungal resistance and the presence of specific virulence traits and genes suggesting that virulence characteristics and resistance profiles are interrelated. 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.

Declarations:

Conflicts of interest: There is not 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.

REFERENCES

1. Cavalheiro M., Teixeira M.C. Candida Biofilms: Threats, challenges, and promising strategies. Front. Med. 2018; 5:28.
2. Sardari, A., Zarrinfar, H., & Mohammadi, R. Detection of ERG11 point mutations in Iranian fluconazole-resistant Candidaalbicans isolates. Current Medical Mycology. 2019; 5(1): 7-14.
3. Spettel K, Barousch W, Makristathis A, et al.: Analysis of antifungal resistance genes in Candidaalbicans and Candidaglabrata using next generation sequencing. PLoS One. 2019; 14(1):e0210397.
4. A.M. Borman, E.M. Johnson, Name Changes for Fungi of Medical Importance, 2018 to 2019, J. Clin. Microbiol. 2021; 59: e01811–e01820, https://doi.org/10.1128/ JCM.01811-20.
5. L. Cern ˇ akov ´ a, ´ A. Lí ˇskova, ´ L. Lengyelov´ a, C.F. Rodrigues, Prevalence and antifungal susceptibility profile of Oral Candida spp. isolates from a Hospital in Slovakia, Med. Kaunas Lith. 2022; 58; 576, https://doi.org/10.3390/medicina58050576.
6. B. Benito-Cruz, S. Aranda-Romo, F.J. Lopez-Esqueda, ´ E. de la Rosa-García, R. Rosas-Hernandez, ´ L.O. S´ anchez-Vargas, Oral Candida isolates and fluconazole susceptibility patterns in older Mexican women, Arch. Gerontol. Geriatr. 2016; 65 :204–210.
7. WHO TEAM, WHO Fungal Priority Pathogens List to Guide Research, Development and Public Health Action. https://www.who.int/publications/i/item/9789 240060241, 2022.
8. A.N. R, N.B. Rafiq, Candidiasis, in: StatPearls, StatPearls Publishing, Treasure Island (FL), 2022. http://www.ncbi.nlm.nih.gov/books/NBK560624/ (accessed July 22, 2022).
9. G. Quindos, ´ S. Gil-Alonso, C. Marcos-Arias, E. Sevillano, E. Mateo, N. Jauregizar, E. Eraso, Therapeutic tools for oral candidiasis: current and new antifungal drugs, Med. Oral Patol. Oral Cir. Bucal. 2019; 24 : e172–e180, https://doi.org/10.4317/ medoral.22978.
10. A. Rai, S.R. Misra, S. Panda, G. Sokolowski, L. Mishra, R. Das, B. Lapinska, Nystatin effectiveness in Oral candidiasis treatment: a Systematic Review & Meta-Analysis of clinical trials, Life. 2022; 12 : 1677,
11. S.G. Whaley, E.L. Berkow, J.M. Rybak, A.T. Nishimoto, K.S. Barker, P.D. Rogers, Azole antifungal resistance in Candida albicans and emerging non-albicans Candida species, Front. Microbiol. 2016; 7 ; 2173.
12. I. Chitapanarux, S. Wongsrita, P. Sripan, P. Kongsupapsiri, P. Phakoetsuk, S. Chachvarat, K. Kittidachanan, An underestimated pitfall of oral candidiasis in head and neck cancer patients undergoing radiotherapy: an observation study, BMC Oral Health . 2021; 21 ;353.
13. A.L. Jayachandran, R. Katragadda, R. Thyagarajan, L. Vajravelu, S. Manikesi, S. Kaliappan, B. Jayachandran, Oral candidiasis among Cancer patients attending a tertiary Care Hospital in Chennai, South India: an evaluation of Clinicomycological association and antifungal susceptibility pattern, Can. J. Infect. Dis. Med. Microbiol. J. Can. Mal. Infect. Microbiol. Medicale. 2016: 8758461, https:// doi.org/10.1155/2016/8758461.
14. M. Jain, R. Shah, B. Chandolia, A. Mathur, Y. Chauhan, J. Chawda, S. Mosby, S. Bhagalia, The Oral carriage of Candida in Oral Cancer patients of Indian origin undergoing radiotherapy and/or chemotherapy, J. Clin. Diagn. Res. JCDR. 2016; 10 ZC17–20, https://doi.org/10.7860/JCDR/2016/15702.7180.
15. M.H. Al-Yasiri, A.-C. Normand, C. L'Ollivier, L. Lachaud, N. Bourgeois, S. Rebaudet, R. Piarroux, J.-F. Mauffrey, S. Ranque, Opportunistic fungal pathogen Candida glabrata circulates between humans and yellow-legged gulls, Sci. Rep. 2016; 6 36157, https://doi.org/10.1038/srep36157.
16.] A. Talukdar, R. Barman, A. Sarma, M. Krishnatreya, J. Sharma, A. Kataki, Fungal profile and antifungal susceptibility pattern of candidiasis in esophageal cancer patients, J. Cancer Res. Ther. 2020; 16 : 209, https://doi.org/10.4103/jcrt.JCRT_ 581_18.
17.] M. Taylor, M. Brizuela, A. Raja, Oral candidiasis, in: StatPearls, StatPearls Publishing, Treasure Island (FL), 2023. http://www.ncbi.nlm.nih.gov/books/N BK545282/.

18. D.D.S.C.M. Castelo-Branco, M.D.A.N. Paiva, C.E.C. Teixeira, E.P. ´ Caetano, G.M.D. M. Guedes, R.D.A. Cordeiro, R.S.N. Brilhante, M.F.G. Rocha, J.J.C. Sidrim, Azole resistance in Candida from animals calls for the one health approach to tackle the emergence of antimicrobial resistance, Med. Mycol. 2020; 58: 896–905, https:// doi.org/10.1093/mmy/myz135.
19. A.K. Urbanek, Z. Łapinska, ´ D. Derkacz, A. Krasowska, The role of ERG11 point mutations in the resistance of Candida albicans to fluconazole in the presence of lactate, Pathog. Basel Switz. 2022; 11 1289, https://doi.org/10.3390/ pathogens11111289.
20. A.S. Odiba, O.A. Durojaye, I.M. Ezeonu, A.C. Mgbeahuruike, B.C. Nwanguma, A new variant of mutational and polymorphic signatures in the ERG11 gene of fluconazole-resistant Candida albicans, Infect. Drug Resist. 15 (2022) 3111–3133,
21. Ying Peng et al. Cryo-EM structures of *Candida albicans* Cdr1 reveal azole-substrate recognition and inhibitor blocking mechanisms. 2024; *Nature Communications* volume 15, Article number: 7722
22. Aida Esfahani et al. Up-regulation of *CDR1* and *MDR1* efflux pump genes and fluconazole resistance are involved in recurrence in *Candida albicans*-induced vulvovaginal candidiasis. Diagnostic Microbiology and Infectious Disease. 2024; 109(1): 116242
23. Clinical and Laboratory Standards Institute (CLSI).Reference method for broth dilution antifungal susceptibility testing of yeasts: approved standard, 3rd ed. CLSI document M27-A3. Clinical and Laboratory Standards Institute, Wayne, PA. 2024.
24. Mohammed A. El-Kholy et al. Analysis of CDR1 and MDR1 Gene Expression and ERG11 Substitutions in Clinical Candida tropicalis Isolates from Alexandria, Egypt. Brazilian Journal of Microbiology. 2023; 54:2609–2615
25. Ben-Ami R, Olshtain-Pops K, Krieger M, et al. Resistance mechanisms in Candida tropicalis: an emerging challenge. J Clin Microbiol. 2012; 50(11):3435–3442.
26. Vignesh Kanna B. et al. Isolation and identification of candida species from various clinical samples in a tertiary care hospital. International Journal of Research in Medical Sciences. 2017; 5 (8).
27. Sharma, Shweta R. et al. "Distribution Of Candida Infection In Clinical Samples And Their Antifungal Susceptibility Pattern In Hospital Of Western U.P.2023.
28. Murray MP, Zinchuk R, Larone DH. CHROM agar Candida as the sole primary medium for isolation of yeast and as a source medium for the rapid-assimilation of trehalose test. J Clin Microbiol. 2005; 43:1210-2.
29. Murray MP, Zinchuk R, Larone DH. CHROM agar Candida as the sole primary medium for isolation of yeast and as a source medium for the rapid-assimilation of trehalose test. J Clin Microbiol. 2005; 43:1210-2
30. Golia S, Reddy KM, Karjigi KS, Hittinahalli V. Speciation of Candida using chromogenic and cornmeal agar with determination of fluconazole sensitivity. Al Ameen J Med Sci. 2013; 6(2):163-6
31. Vijaya D, Harsha TR, Nagaratanamma T. Candidaspeciation using CHROM agar. J Clin Diagn Res. 2011; 5(4):755-7.
32. Adhikary R, Joshi S. Species distribution and anti-fungal susceptibility of candidemiaat a multi super-speciality center in Southern India. Ind J Med Microbiol. 2013; 29:309-11.
33. Ben-Ami R, Olshtain-Pops K, Krieger M, et al. Resistance mechanisms in Candida tropicalis: an emerging challenge. J Clin Microbiol. 2012; 50(11):3435–3442.
34. .Coste A, Selmecki A, Forche A, et al. CDR1 and azole resistance in Candida albicans. Eukaryot Cell. 2007; 6(4):678–685.
35. Papon N, Noël T, Florent M, et al. ERG11 gene and azole resistance in Candida species: a molecular perspective. Antimicrob Agents Chemother. 2013; 57(6):2693–2701.
36. 5. Su L, Li Y, Liu Y, Ma R, Liu Y, Huang F, et al. Antifungal-inbuilt metal–organic-frameworks eradicate candida albicans biofilms. Adv Funct Mater 2020; 30:2000537. doi: 10.1002/adfm.202202895.

37. Mohammed A. El-Kholy et al. Analysis of *CDR1* and *MDR1* Gene Expression and *ERG11* Substitutions in Clinical *Candida tropicalis* Isolates from Alexandria, Egypt. 2023; 54: 2609-2615.
38. Mohaddeseh Larypoor et al. Comparison of expression level of CDR1 and MDR1 genes in stages of biofilm formation of Candida albicans and Candida tropicalis. Journal of Ilam University of Medical Sciences 2024; 32(3): 47-64