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To Study The Vulvovaginal Candidiasis in Pregnancy With Special Reference to Cdr1 Gene in Women's Attending a Tertiary Care Centre

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ABSTRACT

INTRODUCTION: Candida is the leading cause of vaginitis, and 75% of women have at least one episode in their lifetimes. Candida colonisation in vagina was reported to be 20 %, rising to 30 % during pregnancy. Studies show that pregnant women with VVC are more likely than healthy women to have it. Both symptomatic and asymptomatic candidal colonisation rises throughout pregnancy. It is unknown, therefore, how strains that produce silent infection vary from those that cause symptomatic infection.
AIM AND OBJECTIVE: To study the vulvovaginal candidiasis in pregnancy with special reference to CDR1 gene in women's attending a tertiary care centre.
MATERIAL AND METHODS: This was a cross sectional study conducted in the Department of Microbiology at a tertiary care centre. A total of 962 clinical 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 CDR1 was detected using the PCR.
RESULTS: In the present study out of 962 clinical samples, 51.1% (492) were culture positive, among them 28% (138) were Candida isolates. Out of which 53(38.4%) were Candida albicans while 85(61.6%) were Non-albicans Candida. Among Non-albicans Candida,the frequency of Candida tropicalis was observed to be maximum with urine samples and least for ET secreation. It was observed that the maximum sensitivity was observed with Amphotericin-B (95%), followed by Voriconazole(85%) and itraconazole (49.2%). The prevalence of CDR1 expression was 4.3% among Candida spp.
CONCLUSION: Characterising the virulence factors in Candida species that have been isolated from a range of clinical specimens will help us better understand the pathophysiology and clinical outcomes of candidiasis. Further research is required to improve patient outcomes in clinical practice and address the evolving issues brought on by Candida infections. By considering the interplay of genetic changes, phenotypic characteristics, and patient-related factors, azole resistance can be evaluated more thoroughly.

Keywords: Molecular Characterization, Virulence factors, Fluconazole, DNA, PCR, CDR1, Gene Expression

1. INTRODUCTION

Candida is an integral part of the intricate vaginal microflora, but under certain pathological conditions, it can lead to Vulvovaginal Candidiasis (VVC) [1,2] Although most VVC infections are caused by *Candida albicans*, non-

albicans *Candida* (NAC) infections are on the rise, perhaps as a result of over-the-counter medications and low-dose azole maintenance regimens [2].

A higher role of Candidal vaginal colonisation in women has been associated with a number of risk factors, including a compromised immune system caused by diabetes, obesity, HIV/AIDS, prolonged use of corticosteroids or broad-spectrum antibiotics, physiological conditions such as ageing, pregnancy, use of high-level oestrogen and progesterone pills, use of intrauterine contraceptive devices (IUCDs), diaphragms, condoms, frequent sexual contact, and use of vaginal douche [3,4].

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 [5,6]. It is effective against a wide range of Candida species, including C. *albicans*, C. *glabrata*, C. *tropicalis*, C. *krusei*, and C. *parapsilosis.* Fluconazole resistance is usually a stable phenotype that is maintained in the absence of selection pressure by the drug. This implies that genetic alterations have occurred in the resistant isolates that result in a constitutive overexpression of the drug efflux pumps [7].

Prevalence surveys indicate that about 20% of women have vaginal colonisation of Candida species, and this percentage increases to about 30% during pregnancy. Some studies indicated that pregnant women had a considerably greater risk of asymptomatic Candida vaginal infections, whereas others reported that pregnant women were more likely to have symptomatic infections [8,9]. Whether the Candida that causes VVC in pregnant women is a commensal genitourinary tract infection or a unique virulent strain is unknown [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.

However, the prevalence of drug resistance to fluconazole among *Candida albicans* is an increasing concern in the medical community [14]. 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 [15,16].

C. albicans can develop resistance to fluconazole by different molecular mechanisms, including alterations in the sterol biosynthetic pathway, overexpression of *ERG11*, which encodes the target enzyme of fluconazole (sterol 14α-demethylase, or Erg11p), mutations in *ERG11* that result in a reduced affinity of Erg11p for fluconazole, and overexpression of genes encoding membrane transport proteins (*CDR1*, *CDR2*, and *MDR1*) that actively transport fluconazole out of the cell. 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.

The *MDR1* gene encodes an efflux pump of the major facilitator superfamily, whose members use the proton gradient across the cytoplasmic membrane as an energy source for transport. While *MDR1* is normally expressed only at low levels in standard laboratory media, many fluconazole-resistant clinical *C. albicans* isolates constitutively overexpress *MDR1*. Deletion of the *MDR1* gene from *MDR1*-overexpressing *C. albicans* isolates resulted in decreased fluconazole resistance of the mutants, confirming that *MDR1* overexpression contributed to the resistant phenotype of these isolates [18]. Expression of *MDR1* from a plasmid in the heterologous host *Saccharomyces cerevisiae* resulted in increased resistance of the transformants to fluconazole and a variety of structurally unrelated toxic compounds, demonstrating that *MDR1* encodes a multidrug resistance protein. The 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 .

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 a number of ways to identify Candida species, including molecular techniques like PCR and DNA sequencing, biochemical tests that look at the organism's capacity to ferment particular sugars, produce particular enzymes, or use particular substrates, microscopic examination that looks at its morphological characteristics, and advanced technology like MALDI-TOF MS, which is a quick, precise, and economical way to identify microorganisms.
Overuse of this medication, however, may cause resistance to develop, making the fungus more difficult to cure. Fluconazole should therefore only be used when absolutely required, and other therapies should be used wherever feasible [22]. Given these worries, it's critical to assess the scope of the issue, identify the species of Candida causing infections, and ascertain how susceptible they are to antifungal medications. Furthermore, identifying the CDR1 gene in fluconazole-resistant *Candida albicans* can aid in the development of more potent treatments by shedding light on the mechanisms behind resistance. In order to identify the molecular characteristics of the CDR1 gene and its correlation with antifungal resistance in pathogenic Candida species, the current investigation was conducted.

2. 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, January 2024 to January 2025. The Demographic details and clinical history along with the relevant clinical investigations was recorded after the informed consent.
Inclusion Criteria: Candida isolates from every clinical specimen in pure culture were included in the study
Exclusion Criteria: Isolates of Candida species from mix cultures and repeat isolates from the same clinical specimen of the same patient were excluded.

3. LABORATORY EXAMINATION

Isolation and Identification of *Candida albicans*
Samples- Urine, sputum, blood, vaginal swabs collected using aseptic precautions as per the Standard CLSI guidelines.

4. 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

5. LABORATORY DIAGNOSIS

Direct Analysis Swabs or scraping the afflicted area can be used to obtain clinical specimens from sick skin or nails. The wet mount technique is the recommended approach for direct investigation of clinical specimens from nail and cutaneous candidiasis. Most of the time, specimens must be treated with a keratinolytic, usually 10–30% KOH, to enable microscopic inspection.

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	35
Extension	1 min 30 s	72º C	
Final extension	10 min	72º C	

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

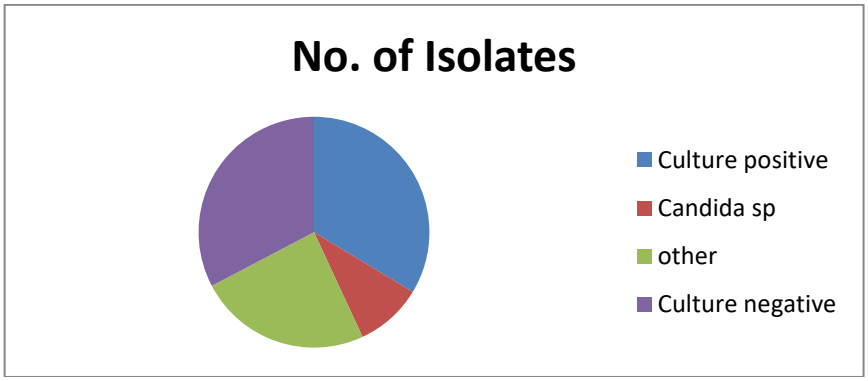
6. RESULTS

In the current study out of the 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.3 : Frequency of culture positive and culture negative.

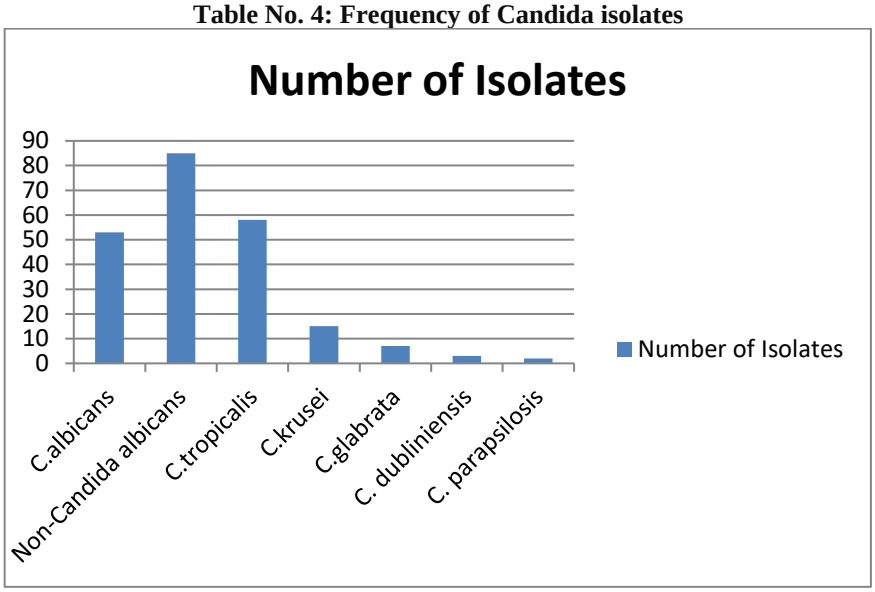
In this table 51.1% were culture positive out of which 28% were Candida isolates.



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. 4: Frequency of Candida isolates



Graph No. 2: Graphical representation of species distribution

From the Table no. 4 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.5 : 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. 6: 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 7: 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	94.2% (130)	5.7% (8)
Amphotericin-B	95.6% (132)	4.3% (6)

Table No. 8: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

SPECIES	TOTAL SAMPLES	CDR1
Candida albicans	**53**	**3**
Candida tropicalis	**58**	**2**
Candida krusei	**15**	**0**
Candida glabrata	**7**	**1**
Other non-*Candida* spp.	**5**	**0**
Total	**138**	**6**

Table No. 9: Gene Distribution of *Candida* and Non-*Candida* Species: *CDR1* gene

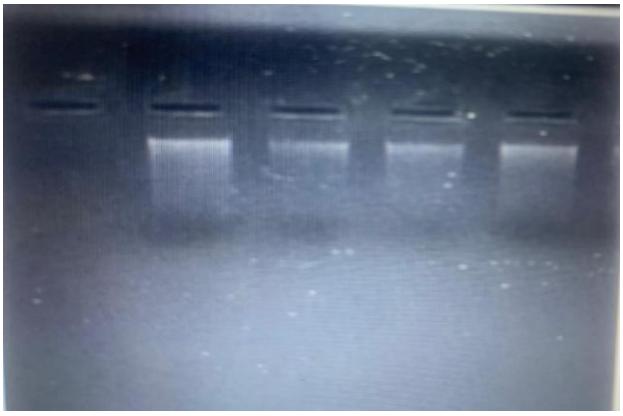


Figure No. 2 : The DNA Extraction in Agarose gel

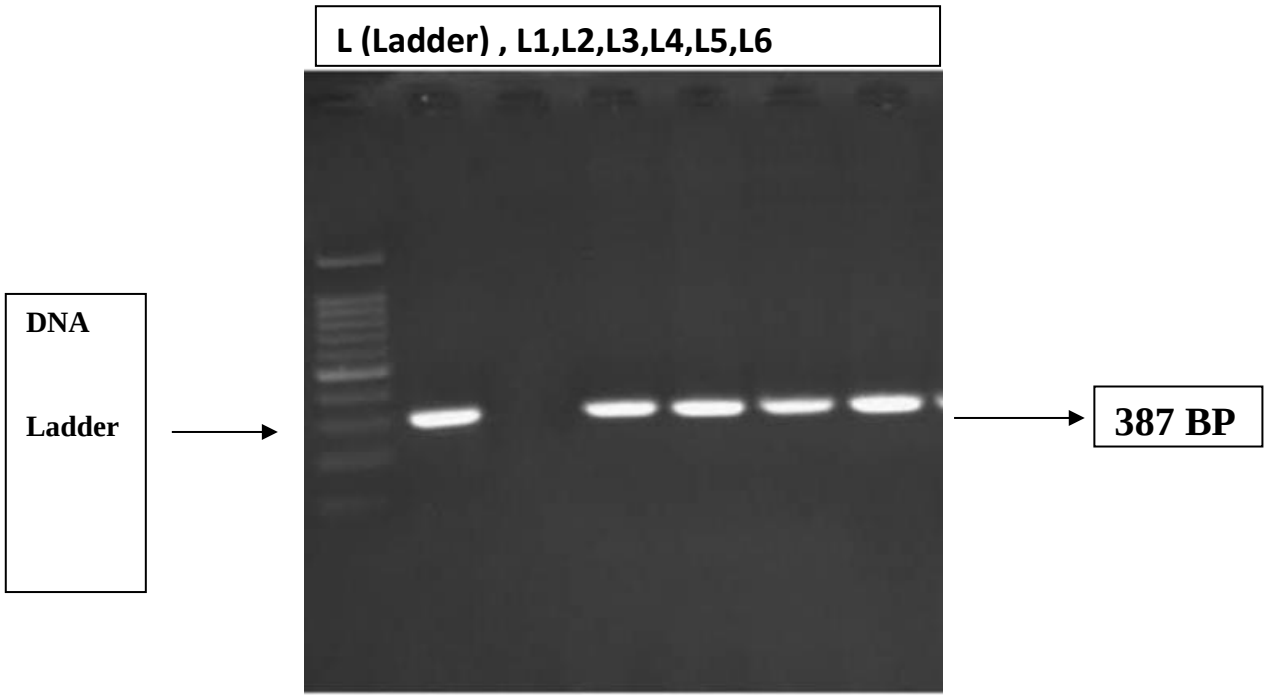


Figure No. 3: The Gene Extraction CDR1 gene

L is the Ladder, L1 corresponds to the Positive Control ATCC CDR1 resistant; L2 corresponds to the Negative control for CDR1; L3- L6 are the sample positive for resistant gene CDR1

GENE	CDR1
Present study	6 (4.3%)

Table No. 10: Distribution of *Candida* Genes

This table reflects the observed prevalence of associated genes in *Candida* in the present study of CDR1 with 4.3%.

7. DISCUSSION

Pregnancy is a predisposing factor for vulvovaginal candidiasis (VVC) . During pregnancy, there is an increase in progesterone and oestrogen levels, especially in the last trimester. Progesterone has an inhibitory effect on the anti-*Candida* activity of neutrophils. On the other hand, oestrogen reduces the ability of vaginal epithelial cells to inhibit the growth of *Candida albicans* on them [3]. However, about 75% of women generally harbour this fungus without it causing harm to them . During normal pregnancy, candidiasis is frequently encountered without significant risk for the foetus. Nevertheless, pregnancy may be negatively affected by VVC [25].

In the current study out of 138 Candida isolates, the 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 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].

Candida is the leading cause of vaginitis, and 75% of women have at least one episode of infection in their lives, with pregnancy being a predisposing factor. If left untreated, vulvovaginal candidiasis (VVC) can lead to chorioamnionitis with subsequent abortion, prematurity and congenital infection of the neonate [36].

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.

If untreated, vaginal candidiasis can lead to chorioamnionitis with subsequent abortion and prematurity in pregnant women, congenital infection of the neonate and pelvic inflammatory disease (PID) resulting in infertility in non-pregnant women .

VVC could be a risk factor for candidemia in preterm neonates during normal pregnancy. Amongst the *Candida* species isolated from vaginal specimens, *C. albicans* is the most predominant, followed by other non-*albicans Candida* (NAC) such as *C. glabrata, C. tropicalis, C. dubliniensis* and *C. krusei* [37,38]. With the increase in frequency of non-*albicans Candida* being isolated from clinical specimens, recent studies indicated that non-*albicans Candida* are now considered pathogens . As a result, compared to *Candida albicans*, NAC are developing resistance to most antifungals used as therapy to treat VVC . This is widely attributed to the use of over the counter (OTC) drugs and empiric regimes to treat these infections, since speciation and antifungal susceptibility testing of *Candida* isolates are not done routinely for clinical purposes.

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 [39].

8. CONCLUSION

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.In order to better treat Candida infections, this emphasises the necessity of tailored antifungal medication and additional investigation into the processes that connect virulence factors with drug resistance.

9. 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] J.B. Mullick *et al.* Changing trends of Candida isolates and their antifungal susceptibility pattern in vulvovaginal candidiasis cases of Tripura, North East India J Evol Med Dent Sci. 2015

[2] Ahmad A, Khan AU. Prevalence of Candida species and potential risk factors for vulvovaginal candidiasis in Aligarh, India. Eur J Obstet Gynecol Reprod Biol. 2009; 144(1):68–71.

[3] Góralska K, Szybka M, Karuga FF, Pastuszak-Lewandoska D, Brzeziańska-Lasota E. Acquired resistance or tolerance? - in search of mechanisms underlying changes in the resistance profile of Candida albicans and Candida parapsilosis as a result of exposure to methotrexate. J Mycol Med. 2024 Jun;34(2):101476.

[4] A.M. Borman, E.M. Johnson, Name Changes for Fungi of Medical Importance, 2018 to 2019, J. Clin. Microbiol. 2021; 59: e01811–e01820.

[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.

[6] Bauters TG, Dhont MA, Temmerman MI, Nelis HJ. Prevalence of vulvovaginal candidiasis and susceptibility to fluconazole in women. Am J Obstet Gynecol. 2002; 187(3):569–74.

[7] Esfahani A, Omran AN, Salehi Z, Shams-Ghahfarokhi M, Ghane M, Eybpoosh S, Razzaghi-Abyaneh M. Molecular epidemiology, antifungal susceptibility, and ERG11 gene mutation of Candida species isolated from vulvovaginal candidiasis: Comparison between recurrent and non-recurrent infections. Microb Pathog. 2022 Sep;170:105696.

[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] Nelson M, Wanjiru W, Margaret MW. Prevalence of vaginal candidiasis and determination of the occurrence of Candida species in pregnant women attending the antenatal clinic of Thika District hospital. Kenya Open Journal of Medical Microbiology. 2013; 3(4):264.

[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] Banerjee A, Pata J, Sharma S, Monk BC, Falson P, Prasad R. Directed mutational strategies reveal drug binding and transport by the MDR transporters of Candida albicans. J Fungi . 2021; 7(2):68.

[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] Mehta A, Kumar M, Bhumbla U, Vyas A, Dalal A. Comparison of different media for germ tube production by Candida albicans: a retrospective study. Int J Curr Microbiol App Sci. 2018; 7(6):819–823

[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] Castanheira M, Deshpande LM, Messer SA, Rhomberg PR, Pfaller MA. Analysis of global antifungal surveillance results reveals predominance of Erg11 Y132F alteration among azole-resistant Candida parapsilosis and Candida tropicalis and country-specific isolate dissemination. Int J Antimicrob Agents. 2020 Jan;55(1):105799. doi: 10.1016/j.ijantimicag.2019.09.003.

[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] Oviasogie F, Okungbowa F. Candida species amongst pregnant women in Benin city, Nigeria: effect of predisposing factors. Afr J Clin Exp Microbiol. 2009; 10(2):92–8.

[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] Holzer I, Farr A, Kiss H, Hagmann M, Petricevic L. The colonization with Candida species is more harmful in the second trimester of pregnancy. Arch Gynecol Obstet. 2017; 295(4):891–5.

[37] Mandras N, Tullio V, Allizond V, Scalas D, Banche G, Roana J, Robbiano F, Fucale G, Malabaila A, Cuffini AM, Carlone N. In vitro activities of fluconazole and voricanazole against clinical isolates of Candida spp. determined by disk diffusion testing in Turin, Italy. Antimicrob Agents Chemother. 2009;53(4):1657–9.

[38] Fan S, Liu X. In vitro fluconazole and nystatin susceptibility and clinical outcome in complicated vulvovaginal candidosis. Mycoses. 2011; 54(6):501–5.

[39] 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