1 **TO STUDY PHENOTYPIC IDENTIFICATION OF CANDIDA**
2 **ISOLATES AMONG CLINICAL SAMPLES AT TERTIARY LEVEL**
3 **HOSPITAL, WESTERN RAJASTHAN.**

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5

6 ## Abstract:

7 **Background:** Candida species are important opportunistic fungal pathogens
8 responsible for a wide spectrum of infections ranging from superficial
9 mucosal disease to invasive candidiasis. Accurate and timely species
10 identification is essential because different Candida species exhibit varying
11 antifungal susceptibility patterns. The increasing occurrence of non-albicans
12 Candida species has further emphasized the need for reliable identification
13 methods.

14 **Objectives:** To study the phenotypic identification of Candida isolates using
15 Sabouraud Dextrose Agar (SDA), Candichrome agar, and Matrix-Assisted
16 Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-
17 TOF MS), and to evaluate their demographic and sample-wise distribution
18 among clinical specimens.

19 **Materials and Methods:** A hospital-based cross-sectional observational
20 study was conducted in the Department of Microbiology, Dr. S.N. Medical
21 College, Jodhpur, from February 2025 to May 2025. A total of 400 clinical
22 specimens including sputum, urine, blood, endotracheal aspirates (ETA),
23 pus, and cerebrospinal fluid (CSF) were processed. Candida isolates were
24 identified by conventional phenotypic methods and confirmed by MALDI-
25 TOF MS.

26 **Results:** Of the 400 clinical samples processed, 74 (18.5%) yielded Candida
27 isolates. Male patients constituted 55.41% of cases, while 44.59% were
28 females. Urban residents accounted for 58.11% of isolates. Most cases were
29 obtained from hospitalized patients (71.62%). The highest frequency of
30 isolation was observed in children ≤10 years (33.78%) and adults >60 years
31 (27.02%). Sputum was the most common source of Candida isolates
32 (29.72%), followed by urine and ETA (20.27% each). Phenotypic
33 identification and MALDI-TOF MS revealed *Candida albicans* as the
34 predominant species (62.16%), followed by *Candida tropicalis* (29.72%).
35 Less frequently isolated species included *Candida kefyr* (2.70%), *Candida*

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glabrata (2.70%), *Candida parapsilosis* (1.35%), and *Candida krusei* (1.35%).

Conclusion:*Candida albicans* remained the leading species isolated from clinical samples, although non-albicans Candida species constituted a substantial proportion of isolates. MALDI-TOF MS proved to be a rapid and accurate method for species-level identification and can serve as an effective adjunct to conventional phenotypic techniques. Continuous surveillance of Candida species distribution is essential for guiding appropriate antifungal therapy and improving patient outcomes.

Keywords: Candida, Candidiasis, MALDI-TOF MS, Phenotypic Identification, Non-albicans Candida, Candichrome Agar.

Introduction

Candida species are ubiquitous fungi that can exist as commensals in the human body but have the potential to cause a range of infections, collectively termed candidiasis. These infections can vary from superficial mucocutaneous conditions to invasive systemic diseases, particularly in immunocompromised individuals[1]. The increasing prevalence of candidemia, a bloodstream infection caused by *Candida* species, is a significant concern in healthcare settings due to its association with high morbidity and mortality rates. Studies have shown that candidemia accounts for considerable morbidity and mortality, especially among hospitalized patients in critical care units.[1]

Candida species are widespread fungi that commonly exist as commensals within the human microbiota, particularly in the mucosal surfaces of the gastrointestinal and genitourinary tracts.[2] Although generally harmless, these organisms have the capacity to become opportunistic pathogens under certain conditions, especially in individuals with weakened immune systems.

This can result in candidiasis, a spectrum of diseases ranging from superficial mucocutaneous infections to life-threatening systemic conditions such as candidemia, which is a bloodstream infection caused by *Candida*spp.[2] Candidemia has emerged as a serious healthcare-associated infection, particularly among critically ill and hospitalized patients, due to its high rates of morbidity and mortality.

A growing concern in recent years is the shifting epidemiology of candidemia, where non-*albicans Candida* (NAC) species such as *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, and *C. krusei* are being isolated with

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increasing frequency.[1] These NAC species often exhibit reduced susceptibility to standard antifungal drugs, complicating treatment protocols and highlighting the need for accurate, species-level identification.[2] Of particular concern is the emergence of *Candida auris*, a multidrug-resistant yeast capable of causing hospital outbreaks due to its persistence on surfaces and resistance to multiple antifungal classes.[3]

Candida infections are increasing in hospital settings, especially among immunocompromised patientsdue to prolong use of antibiotic, immunosuppressive therapy, disruption of skin or mucosal barriers, corticosteroids, chronic illnesses, malignancies, organ transplantation, hemodialysis, premature birth, recent surgery, trauma, and the presence of central vascular catheters. In such cases, the non-specific clinical presentation of fungal infections necessitates early consideration of a fungal etiology to improve patient outcomes.[3]

The accurate and timely identification of *Candida* species is paramount for effective patient management. Different *Candida* species exhibit varying antifungal susceptibility profiles; thus, species-level identification informs appropriate antifungal therapy, which is crucial for improving patient outcomes and reducing mortality[2]. Traditional identification methods, such as culture-based techniques and biochemical assays, are often time-consuming and may lack the specificity required for precise species differentiation.[3]

To address these diagnostic challenges, Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) has revolutionized yeast identification. Unlike conventional culture-based or biochemical techniques, which are time-consuming and less specific, MALDI-TOF MS enables rapid and accurate species-level identification by analyzing the unique protein spectra of yeast like pathogens.[3] Its integration into diagnostic workflows, particularly in tertiary care centers, can significantly enhance timely antifungal therapy which is critical in managing candidemia thus reduce mortality. Furthermore, understanding the local epidemiology of *Candida* infections through preciseidentification aids in monitoring trends, detecting outbreaks, and formulating effective infection control policies.

Material and methods

This study was done for a period of 4 Months i.e. Febuary 2025 to May 2025.

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Type of Study: This was a cross-sectional observational hospital-based study.

Sample Size: Total 400 clinical samples were studied.

Source of Data: This study was done in department of microbiology Dr. S.N. Medical College, Jodhpur and their associated groups of hospitals.

Inclusion Criteria:

1. IPD or OPD patients of all age groups and genders attending tertiary level hospitals, their clinical samples like sputum, Endotracheal aspirate (ETA), Bronchoalveolar lavage (BAL), urine, pleural fluid, blood andCSF of positive fungal cultue were included.

Exclusion Criteria:

1. Patients who were already on anti-fungal treatments.

Sample collection and processing – All clinical samples like blood, urine, CSF, BAL, Pus, other sterile fluids were received in associated groups of hospitals. As per standard laboratory protocol samples were processedand pure growth yeast isolates were further identified and confirmed by MALDI-TOF MS.

Clinical Sample Collection and Processing

1. **Blood Samples:** Blood was collected aseptically after disinfecting the venipuncture site with 70% alcohol and povidone-iodine. After drying, 1–3 mL of blood from pediatric and 8–10 mL from adult patients were drawn using sterile syringes and inoculated directly into the BACTEC system bottles for microbial detection.
2. **Urine Samples:** Midstream morning urine was collected in sterile containers. In bedridden patients, urine was drawn aseptically from Foley’s catheter ports using sterile syringes and transferred into 4abeled sterile containers.
3. **Pus and Swab Samples:** Samples from infected sites like wounds, throat were taken using sterile swabs after surface cleaning to avoid contamination. Specimens were sent to the lab within 1 hour, or refrigerated at 4°C if delayed (not exceeding 24 hours).
4. **Respiratory Samples:** Sputum was collected early morning before meals after rinsing the mouth. Patients coughed deeply into sterile containers, which were properly 4abeled.

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142 5. **CSF and Pleural Fluid:** CSF was collected in sterile tubes under
143 aseptic conditions and sent immediately. Pleural fluid was aspirated
144 using sterile syringes and placed in anticoagulant-containing tubes for
145 culture.
146 6. **BAL Fluid:** Bronchoalveolar lavage was collected during
147 bronchoscopy under sterile conditions and transported immediately to
148 the microbiology lab.
149
150

151 **Specimen Transport and Storage**

152 All clinical samples were transported under controlled conditions
153 immediately after collection to ensure timely and accurate microbiological
154 diagnosis. If immediate processing was not feasible, samples were stored
155 under appropriate conditions to preserve their integrity and prevent
156 contamination or degradation.

157 **Identification Techniques**

158 ***Direct Microscopy***

159 **Gram Staining**

160 **Principle:**
161 Gram staining is based on the ability of bacterial cells to retain certain dyes
162 depending on the structural composition of their cell walls. Gram-positive
163 bacteria possess a thick peptidoglycan layer, which traps the crystal violet-
164 iodinecomplex, resulting in a purple appearance under the microscope even
165 after alcohol-based decolorization. In contrast, Gram-negative bacteria, with
166 their thin peptidoglycanlayer and outer membrane, lose the initial dye during
167 decolorization and take up the counterstain (usually safranin), appearing pink
168 or red. Though originally intended for bacterial identification, Gram staining
169 is also useful in visualizing yeast cells, which generally appear Gram-
170 positive due to the characteristics of their cell walls.

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Procedure:

1. Prepare a smear on a slide.
2. Heat-fix over a flame.
3. Stain with crystal violet for 1 minute.
4. Then stain with iodine for 1 minute.
5. Decolorization step - wash the smear with ethanol/acetone for 5-10 seconds.
6. Counterstain with safranin for 1 minute.
7. Rinse the slide let it air dry and examine under oil immersion objectives.

Result Interpretation

- Used to visualize yeasts and pseudohyphae ofCandida species**.**
- Presence of pseudohyphae indicates tissue invasion this is highly significant finding on direct grams staining
- Yeasts typically appear Gram-positive (4–8 µm), but over-decolorization may cause a false Gram-negative appearance.

Phenotypic Identification of Yeast Isolates

Culture Media:[12]

A. **SDA (Sabouraud Dextrose Agar):** Supports general fungal growth.

Composition: SDA agar with antibiotic

- Dextrose 40g/L.
- Peptone 10g/L.
- Agar 15g/L.
- pH ~5.6 ±0.2 at 25°C
- **Chloramphenicol** is added to inhibit bacterial growth.

Culture Procedure:

1. Inoculate specimen using sterile loop on Sabouraud Dextrose Agar medium
2. Streak gently for isolated colony growth.
3. Incubate aerobically at 25°C or 37° for 24-48 hours.
4. Observe colony characteristics – color, size, texture, and pigment**.**

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Species	Colony characterstics of Candida species on SDA agar
Candida albicans	Convex, shiny, creamy
Candida tropicalis	Convex, shiny, creamy
Candida pseudotropicalis	Convex, hard, ivory coloured with mycelia fringe
Candida krusei	Flat, dry
Candida parapsilosis	Wrinkled
Candida gullermondii	Grayish pimk flat, shiny

206

207 B. **CandiChrome Agar:** Chromogenic medium that differentiates
208 Candida spp. Based on colony color.

209 **CandiChrome Agar Testing**

210 **Principle:**CandiChrome Agar is a fast and efficient plate-based method used
211 for the simultaneous isolation and preliminary identification of different
212 *Candida* species. This modern medium differentiates *Candida* species based
213 on distinct colony colors produced through specific biochemical reactions. It
214 enables the identification of multiple *Candida* types—such as *C. albicans*, *C.*
215 *krusei*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. dubliniensis* and other
216 yeast-like organisms in a single test.

217 **Composition:**

218 • Peptone.
219 • Dextrose
220 • chromogenic substrates
221 • agar.

222 **Procedure:**

223 1. Inoculate fresh culture isolate on plate using sterile inoculating loop.
224 2. Streak in zig-zag method on candichrome agar medium
225 3. Incubate at 25–30°C for 24–48 hours.
226 *4.* Observe colony color and morphologyto presumptively identify
227 *Candida species*

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Species	Colony Characterstics on Candi Chrome of Candida species
Candida albicans	Apple green colonies, consistent
Candida tropicalis	Dull blue, to purple colour that diffused into surrounding agar plate with pale pink edges.
Candida parapsilosis	White to pale pink colonies
Candida krusei	Large flat, spreading, pale pink colonies with matt surfaces
Candida glabrata	White large glossy pale pink to violet colonies
Candida kefyr	Small pink to purple colonies

230 **Germ Tube Test**

231 **Purpose:** The germ tube test is a rapid method for identifying *Candida*
232 *albicans* and distinguishing it from other *Candida* species. When incubated
233 in serum *C. albicans* forms germ tubes which are elongated outgrowths that
234 originate from yeast cells. These structures are an early indication of hyphal
235 development and are considered a characterstics feature of *Candida*
236 *albicans*.

237 **Procedure:**

238 1. Suspend yeast colony in 0.5 mL of human/fetal serum.
239 2. Incubate at 35–37°C for 2–3 hours (no shaking).
240 3. Prepare a wet mount from yeast serum suspension on a glass slide,
241 examine under 40x objective under microscope.
242 4. **Result:**
243 - *Positive:* Germ tubes without basal constriction indicate
244 *Candida albicans*.
245 - *Negative:* Suggests non albicans Candida species.

246

247 **MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization Time of**
248 **Flight)**[1]

249 **Principle** – MALDI-TOF Mass Spectrometry works by mixing the analyte
250 with a matrix compound and applying it to a target plate. A laser beam
251 ionizes the sample, and the resulting ions are accelerated into a drift tube.
252 The time it takes for ions to reach the detector is measured and used to

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253 determine their mass-to-charge ratio (m/z), allowing for the identification
254 and characterization of the analytes

255 **Requirements –**

256 1. ATCC *Escherichia coli* 8739 strain (as a control) 24 hours fresh
257 sub culture on sheep blood agar plate.
258 2. CHCA matrix (Cyano-4- hydroxycinnamic acid).
259 3. Formic acid.
260 4. Pure and 24 hours fresh Yeast culture isolates.
261 5. MALDI-TOF Slides.
262 6. Pipette (0-10 µl).
263 7. Toothpick for colony picking.

264 **Procedure –**

265 1. **Sample Preparation:** Pick a yeast colony from an SDA agar plate.
266 2. **Formic Acid Treatment:** First, add **0.5 µL of 70% formic acid** to
267 the yeast colony on the target plate. Allow the formic acid to dry
268 completely. This helps to lyse the cells and release intracellular
269 proteins.
270 3. **Matrix Application:** After the formic acid dries, add 1-2 µL of the
271 matrix solution, typically a-Cyano-4- hydroxycinnamic acid (CHCA),
272 and let it dry.
273 4. **Control –** pick a colony from ATCC E.Coli 8973 strain as a control
274 and make a smear on control spot at center.
275 5. **Drying and Crystallization:** Let the sample air dry at room
276 temperature. Proper drying will result in the formation of matrix
277 crystals, which is essential for successful analysis.
278 6. **Loading the Plate:** Insert the prepared target plate into the MALDI-
279 TOF instrument.
280 7. **Result:** MALDI-TOF then analyses the time of flight and gives the
281 organisms identification report by comparing the time of flight data of
282 analysed isolate with its own database.

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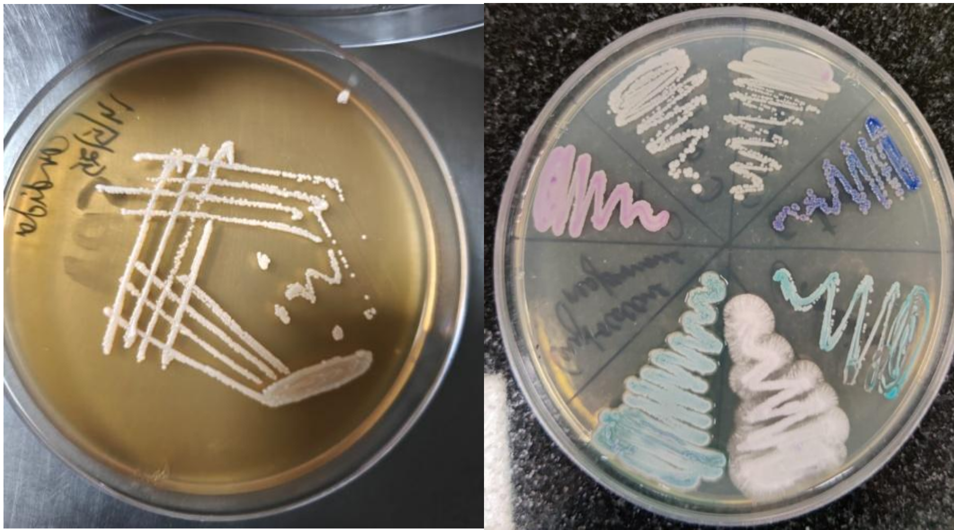

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290 **Figure 1 – *Candia albicans*SDA AgarFigure 2 – Candida species on Candichrome**
291 **agar**
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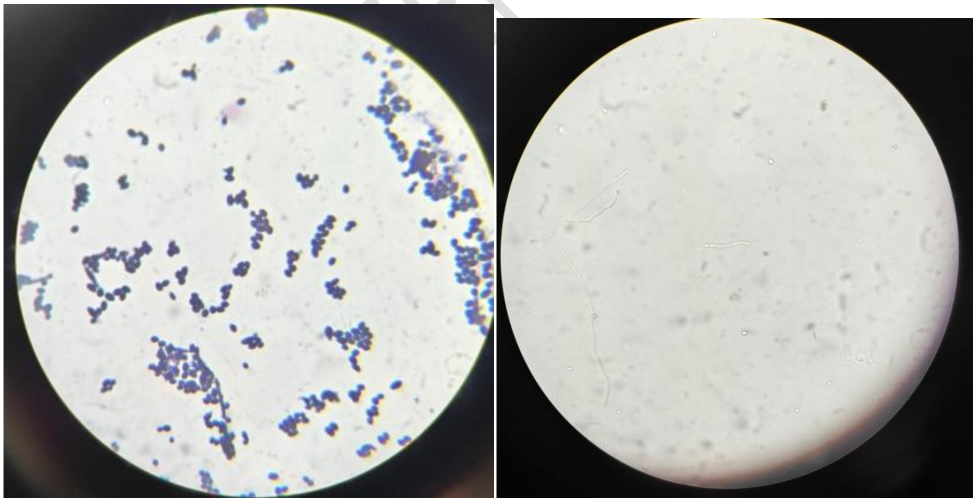

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298 **Figure 3 - Gram's Stain budding yeast cell** **Figure 4 – Germ tube positive**

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Figure 5 – ATCC Esch.coli 8739 strain on sheep blood agar

Figure 6 – MALDI-TOF slide

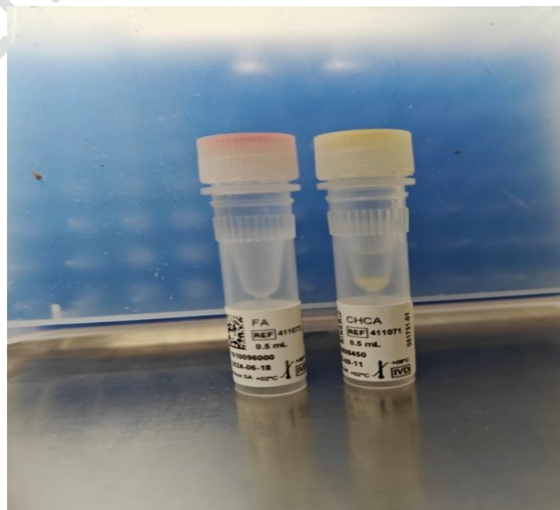

Figure 7 – Matrix (CHACA) & Formic acid

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314 **Figure 8 – Sample processing for MALDI-TOF**

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317 **Figure 9 – Barcode scanner**

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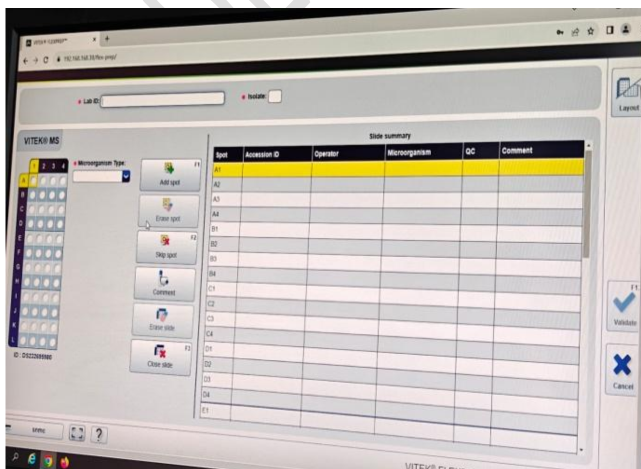
319 

320 **Figure 10 – MALDI-TOF Data entry performa**

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322 **Figure 11 – MALDI-TOF MS**

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325 **Figure 12 – BOD incubator**

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326 ## Result

327 This is the prospective study for a period of 4 months from Feburary to May
328 2025. This study was conducted in the Department of Microbiology at Dr.
329 S.N. Medical College & associated group of hospitals in Jodhpur
330 (Rajasthan).

331

332 **Table-1 Phenotypic identification results of Candida isolates**

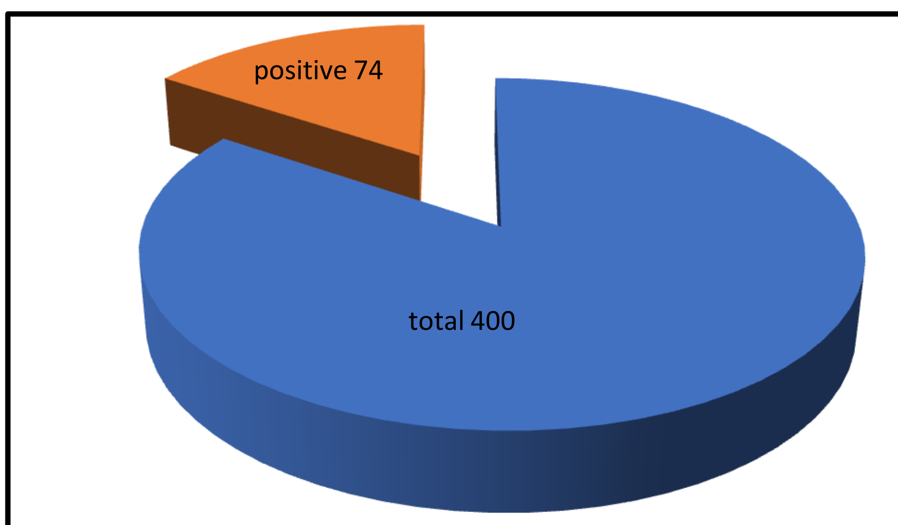
Total	Positive	%
400	74	18.50

333

334 A total of 400 samples were collected and processed for culture during study
335 time period. Out of this 74 (18.50%)samples were positive for Candida
336 isolates, these were studied further.(Table-1)

337

338 **Chart 1 - Phenotypic identification results of Candida isolates**



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342 **Table 2: Gender-wise Distribution of Candida isolates**

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Gender	Number of Cases (N=400)	%
Male	41	55.41
Female	33	44.59
Total	74	100

344 Table 2 Shown gender wise distribution of Candida isolates. Out of 74
345 positive isolates, 41(55.41%) were male and 33(44.59%) were female.

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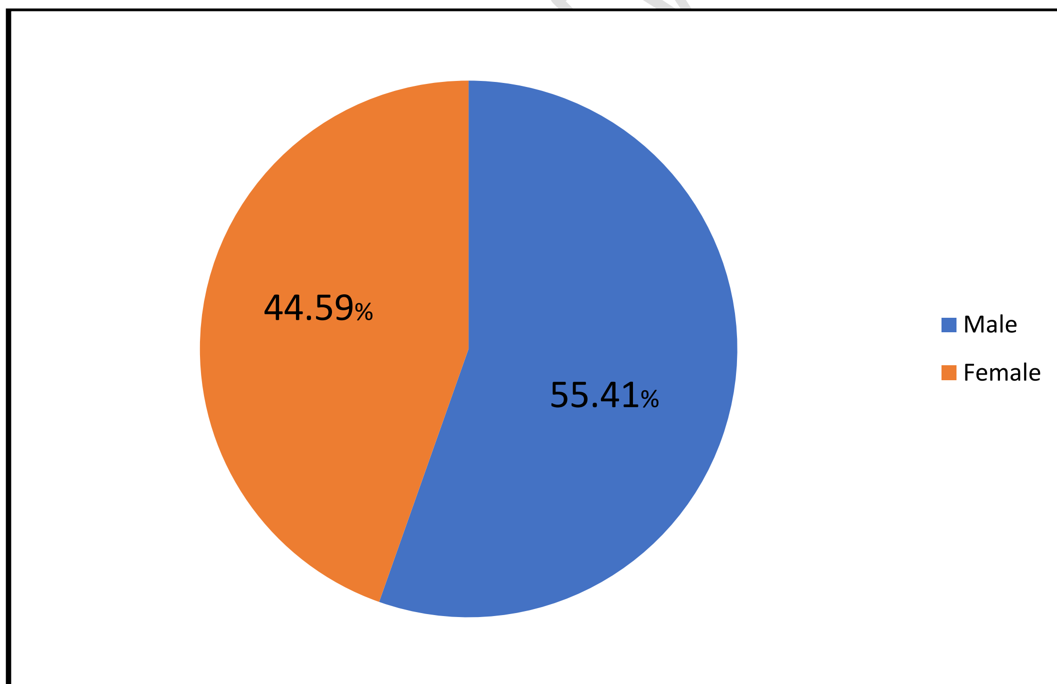
347 **Chart 2 - Gender-wise Distribution of Candida isolates**



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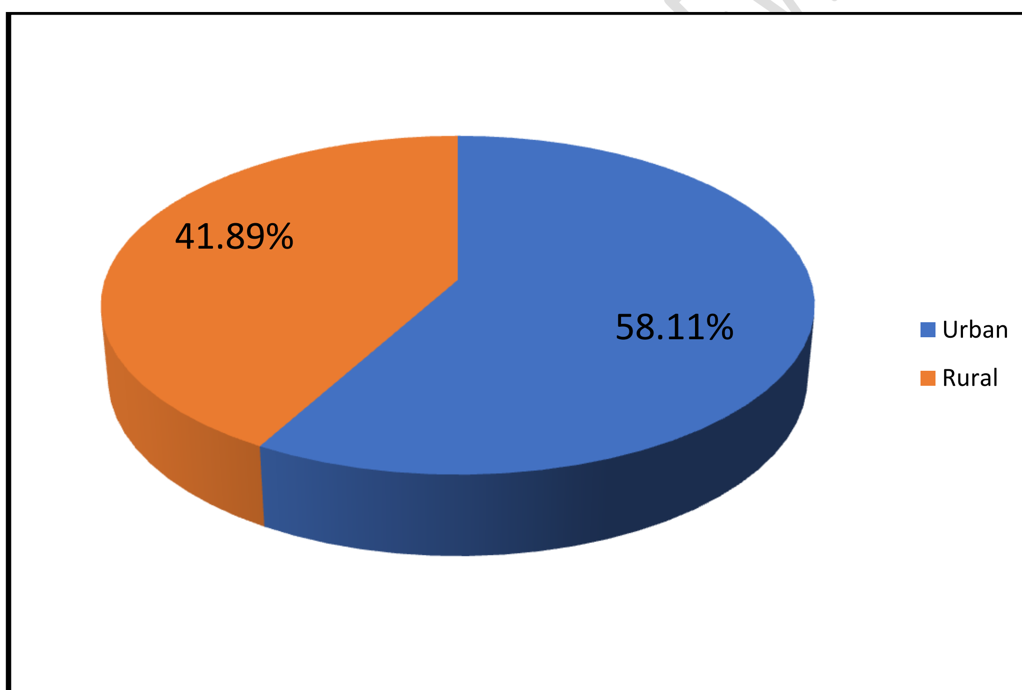
354 **Table 3: Demographic distribution of Candida isolates**

Demography	Number of Cases (N=400)	%
Urban	43	58.11
Rural	31	41.89
Total	74	100

355 Table 3 Shown the demographic distribution of Candida isolates. Out of 74
356 positive isolates, 43(58.11%) were urban and 31(41.89%) were from rural
357 areas.

358

359 **Chart 3 -Demographic distribution of Candida isolates**



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365 **Table 4: OPD and IPD Distribution of Candida isolates**

Department	Number of Cases (N=400)	%
OPD	21	28.38
IPD	53	71.62
Total	74	100

366 As shown in Table 4 out of 74 positive isolates, 21 (23.38) were from OPD
367 and 53(71.62%) were from IPD.This is showing high rate of Candida
368 infection among hospitalized patients.

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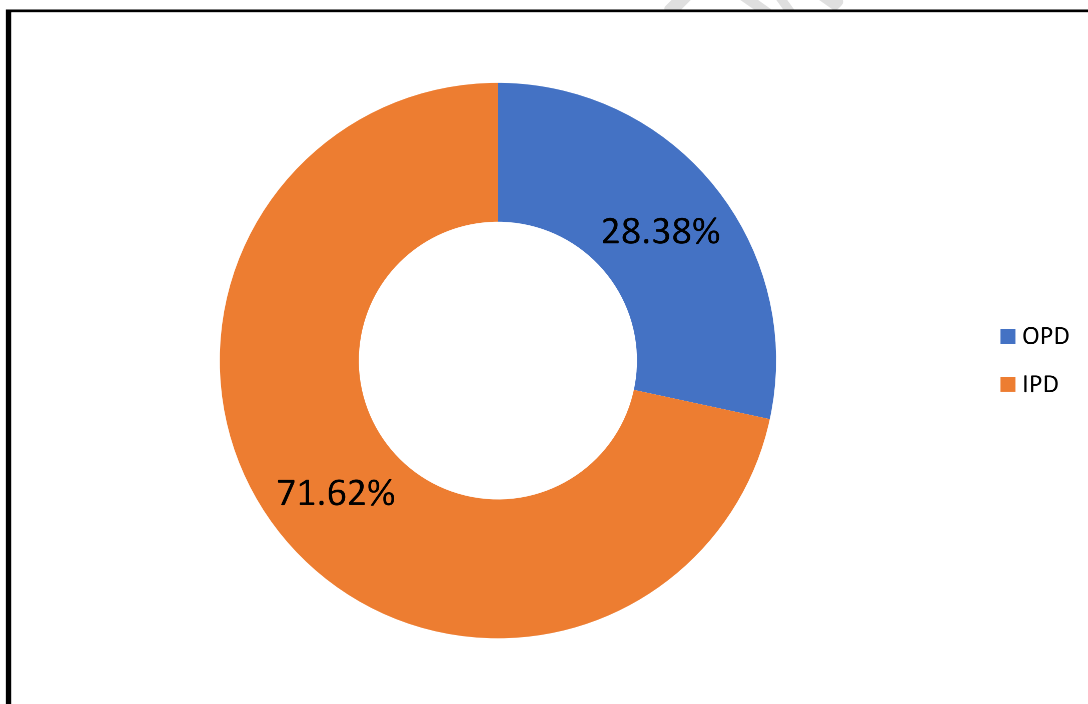
369 **Chart 4 -OPD and IPD Distribution of Candida isolates**



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375 **Table 5:Age wise distribution of Candida isolates**

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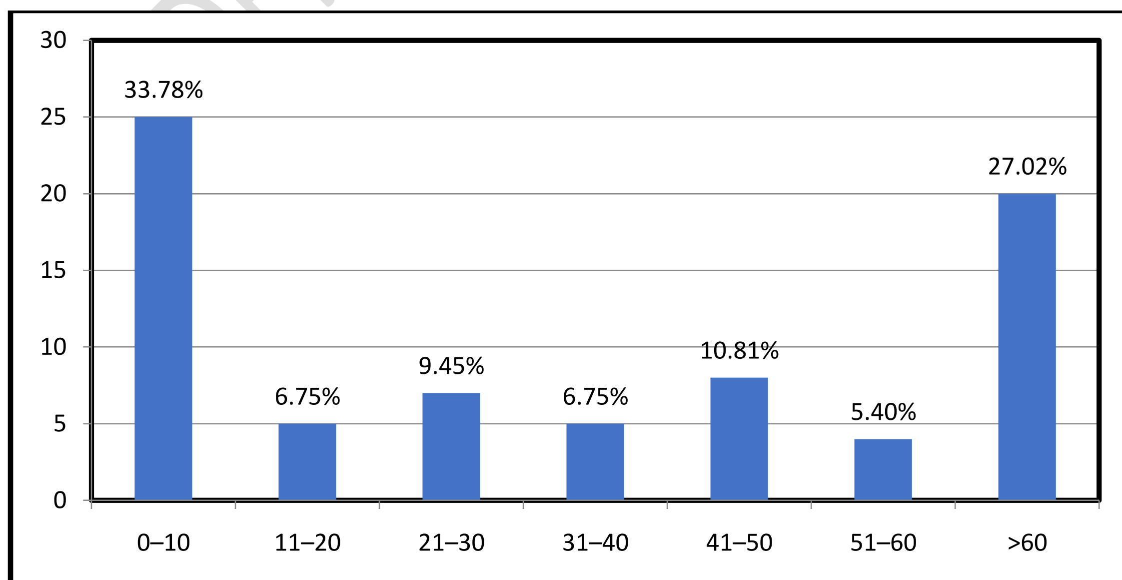
Age Group(in years)	Number of Cases(N=30)	%
≤10	25	33.78
11–20	5	6.75
21–30	7	9.45
31–40	5	6.75
41–50	8	10.81
51–60	4	5.40
>60	20	27.02
Total	74	100

377

378 Table 5 shown Age wise distribution of Candida isolates the maximum
379 positivity was seen in the age group ≤10years (33.78%) followed by >60
380 years (27.02)&41-50 years age group (5.40%).

381

382 **Chart 5 - Age wise distribution of Candida isolates**



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387 **Table 6: Sample-wise Distribution of Candida Species**

Sample type	Total	Positive	%
Sputum	173	22	29.72
Urine	97	15	20.27
Blood	40	11	14.86
ETA	43	15	20.27
Pus	37	10	13.51
CSF	10	1	1.35
Total	400	74	100

388 Table6 presents the distribution of Candida isolates by Sample wise.
389 Maximum cases were seen in sputum(29.72%) followed by urine (20.27%)
390 & ETA (20.27%), blood (14.86%).

391

392 **Chart 6 -Sample-wise Distribution of Candida Species**

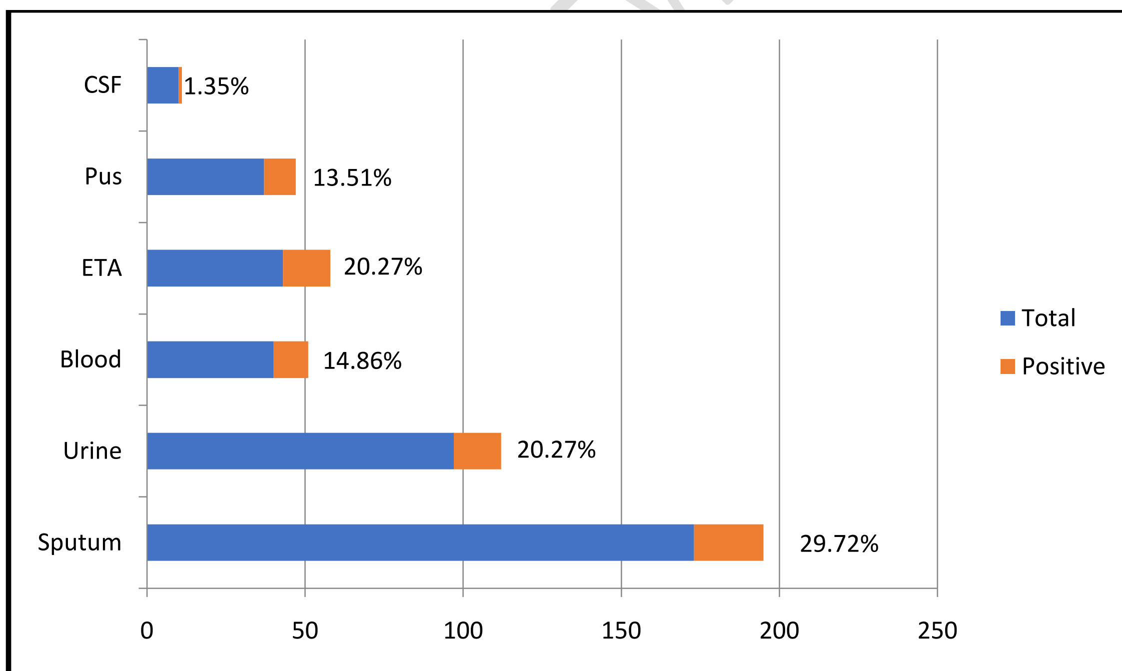

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Table 7. - Phenotypic identification of Candida by growth on Candichrome agar

Species	**Growth Characterstics on Candi-chrome agar**	**N**	**%**
Candida albicans	Apple green colonies,	**46**	**62.16%**
Candida tropicalis	blue, to purple colour colonies	**22**	**29.72%**
Candida kefyr	Small pink colonies	**2**	**2.70%**
Candida glabrata	Small, pink to mauve colonies	**2**	**2.70%**
Candida parapsilosis	White to pale pink colonies	**1**	**1.35%**
Candida krusei	Small pink colonies	**1**	**1.35%**

This table presents the phenotypic identification of *Candida* species based on their characteristic growth and color on Candichrome agar. A total of 74 *Candida* isolates were identified. *Candida albicans* was the most prevalent species, accounting for 62.16% (46 isolates), and was characterized by apple green colonies. The next most common species was *Candida tropicalis*, representing 29.72% (22 isolates), which produced blue to purple colonies. Other less frequently identified species included *Candida kefyr* (2 isolates, 2.70%) and *Candida glabrata* (2 isolates, 2.70%), both forming small pink to mauve colonies. *Candida parapsilosis* (1 isolate, 1.35%) displayed white to pale pink colonies, and *Candida krusei* (1 isolate, 1.35%) also presented as small pink colonies

Chart 7 Phenotypic identification of Candida by growth on Candichrome agar

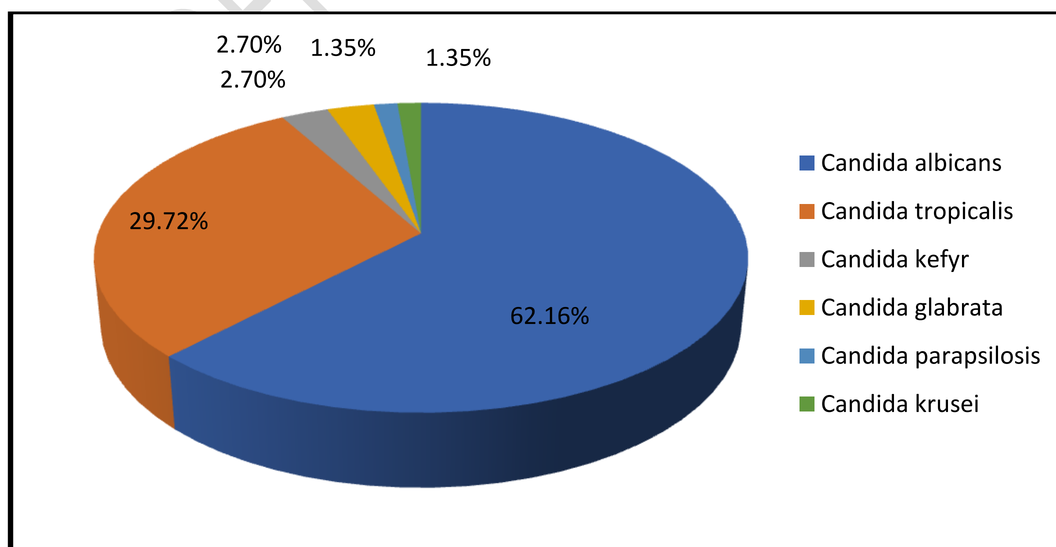

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Table:8 Species wise distribution of Candida isolates by MALDI-TOF

Species	*Candida albicans*		*Candida tropicalis*		*Candida kefyr*		*Candida glabrata*		*Candida parapsilosis*		*Candida krusei*		Total	
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Sputum	18	81.81%	4	18.18%	0	0%	0	0%	0	0%	0	0%	**22**	**29.72%**
Urine	7	46.66%	5	33.33%	1	6.66%	2	13.33%	0	0%	0	0%	**15**	**20.27%**
Blood	6	54.54%	4	36.36%	0	0%	0	0%	0	0%	1	9.09%	**11**	**14.86%**
ETA	9	60%	5	33.33%	1	6.66%	0	0%	0	0%	0	0%	**15**	**20.27%**
Pus	5	50%	4	40%	0	0%	0	0%	1	10%	0	0%	**10**	**13.52%**
CSF	1	100%	0	0%	0	0%	0	0%	0	0%	0	0%	**1**	**1.36%**
Total	**46**		**22**		**2**		**2**		**1**		**1**		**74**	**100%**

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This table provides a detailed breakdown of *Candida* species distribution across various clinical sample types, identified using MALDI-TOF. A total of 74 *Candida* isolates were analyzed.

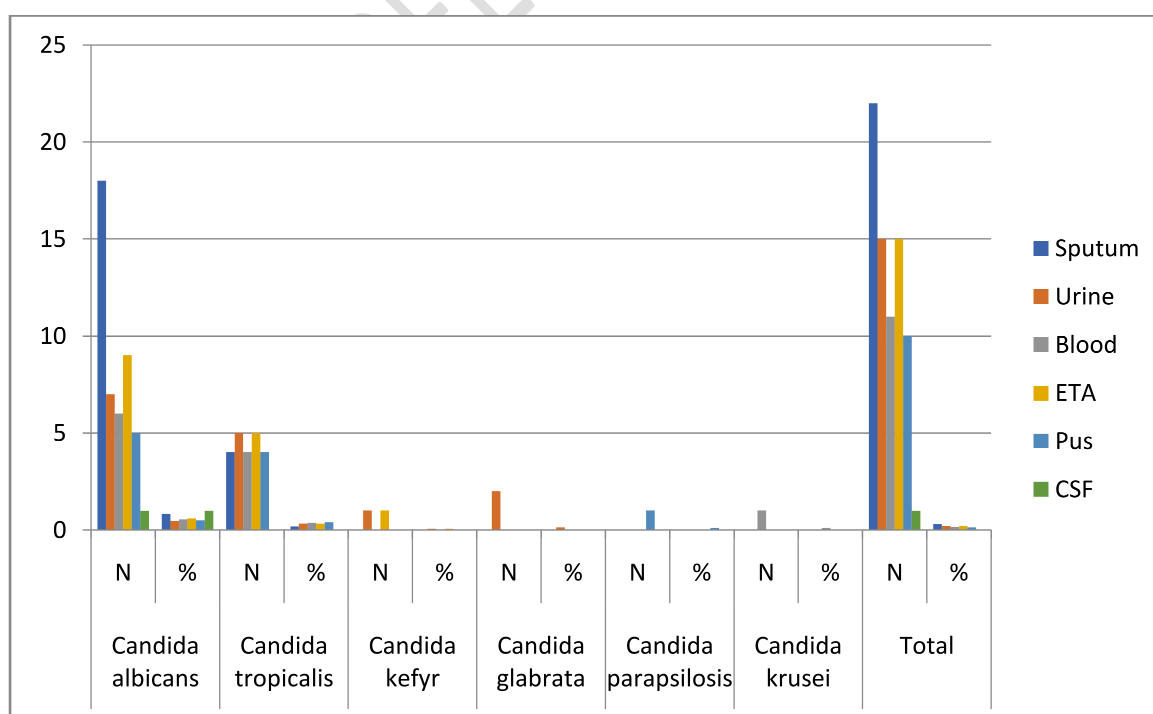
Candida albicans was the predominant species across most sample types, representing 81.81% (18 isolates) in sputum, 60% (9 isolates) in ETA, and 100% (1 isolate) in CSF. It also formed a significant proportion in blood (54.54%, 6 isolates) and pus (50%, 5 isolates).

Candida tropicalis was the second most common species, showing notable presence in sputum (18.18%, 4 isolates), urine (33.33%, 5 isolates), blood (36.36%, 4 isolates), ETA (33.33%, 5 isolates), and pus (40%, 4 isolates).

Other *Candida* species were detected in smaller numbers. *Candida kefyr* was found in urine (1 isolate, 6.66%) and ETA (1 isolate, 6.66%). *Candida glabrata* was identified in urine (2 isolates, 13.33%). *Candida parapsilosis* was present in pus (1 isolate, 10%), and *Candida krusei* was detected in blood (1 isolate, 9.09%).

Overall, sputum samples yielded the highest number of isolates (22, 29.72%), followed by urine (15, 20.27%) and ETA (15, 20.27%). Blood samples accounted for 11 isolates (14.86%), while pus samples contributed 10 isolates (13.52%). A single isolate was found in CSF (1.36%).

Chart 8 -Species wise distribution of Candida isolates by MALDI-TOF



Discussion

The present cross-sectional study, conducted at Dr. S.N. Medical College, Jodhpur, aimed to investigate the phenotypic identification and species distribution of Candida isolates using both conventional methods and MALDI-TOF MS. This analysis sheds light on the evolving epidemiology of candidiasis, particularly in Western Rajasthan.

Out of 400 clinical samples**,** 74 cases (18.5%) tested positive for Candida infections (Table 1). However, other studies like Agarwal et al. [8] found positivity rate of 25% and Singh et al.[25] recorded lower prevelance rate of between 10% and 20%. The variation in the finding could be influenced by factors such as local healthcare practices, environmental conditions, and population vulnerability.

Gender wise Distribution of Candida isolates:

The gender distribution in this study showed a male predominance (55.41%), aligning with the findings of Rajni et al., who suggested that males may have greater exposure to risk factors like catheterization or underlying comorbidities such as diabetes [2]. Urban cases (58.11%) were more prevalent than rural ones (41.89%), possibly due to environmental and occupational exposure, our results emphasize the need for awareness and public health strategies inboth urban and rural populations to effectively address the fungal disease burden.

IPD and OPD wise distribution of Candida isolates:

A significant majority (71.62%) of Candida-positive cases were detected in hospitalized (IPD) patients, affirming findings by Rudramurthy et al. and Mellinghoff et al., who documented elevated candidiasis prevalence among ICU and hospitalized populations due to immunosuppressive therapies, invasive procedures, and prolonged hospital stays [2,4]. Such patients are often subject to multiple risk factors, including prior antibiotic use, steroid therapy, and the presence of indwelling medical devices, which facilitate Candida colonization and infection.

Age group wise distribution of Candida isolates:

A bimodal age distribution was noted, with the highest incidence in the pediatric age group (<10 years, 33.78%) and older adults (>60 years, 27.02%). While candidiasis is typically more common in the elderly due to immunocompromise status and chronic illness, the spike in pediatric cases may reflect heightened diagnostic scrutiny or underlying pediatric conditions.

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Similar age-related vulnerability was noted in studies by Agarwaland Chakrabarti,[2] who reported increased incidence among older adults.[5]

Sample-wise Distribution of *Candida* Isolates:

Table 6 highlights the distribution of *Candida* isolates across different sample types. Out of 400 total clinical samples, 74 (18.5%) were positive for *Candida*. Sputum samples yielded the highest positivity rate (29.72%, 22 isolates), followed by urine and ETA (Endotracheal Aspirate) samples, both contributing 20.27% (15 isolates each). Blood samples accounted for 14.86% (11 isolates), while pus samples comprised 13.51% (10 isolates). Notably, only one *Candida* isolate (1.35%) was detected from CSF. This distribution concordant with findings from other studies in tertiary care settings where respiratory tract samples (sputum, ETA) and urinary samples are frequently implicated in candidiasis, often due to colonization in critically ill or catheterized patients[41,43,29]. The presence of *Candida* in blood and CSF, though less frequent in our study, signifies invasive candidiasis, which is associated with high morbidity and mortality [2,18,32].

Phenotypic Identification of *Candida* Species:

Table 7 illustrates the phenotypic identification of *Candida* species using Candichrome agar. This chromogenic medium allows for presumptive identification based on distinct color production, which is a valuable tool for rapid differentiation in a clinical microbiology laboratory[12]. Our study identified *Candida albicans* as the most prevalent species (62.16%, 46 isolates), characterized by its typical apple green colonies. This finding is consistent with global epidemiology, where *C. albicans* traditionally remains the most common *Candida* species causing human infections.[9,10,11] *Candida tropicalis* was the second most common species (29.72%, 22 isolates), identified by its blue to purple colonies. The increasing prevalence of non-*albicans Candida* (NAC) species, including *C. tropicalis*, *C. glabrata*, and *C. parapsilosis*, has been a growing concern in recent years, as they often exhibit reduced susceptibility to commonly used antifungals[1,5,17]. The detection of *C. kefyr* (2.70%), *C. glabrata* (2.70%), *C. parapsilosis* (1.35%), and *C. krusei* (1.35%) in our study, although in smaller numbers, underscores the importance of accurately identifying these species for guiding appropriate antifungal therapy.[5,6]

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Species-wise Distribution of *Candida* Isolates by MALDI-TOF:

Table 8 provides a more definitive species-level identification using Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS). MALDI-TOF is a rapid, accurate, and cost-effective method for identifying microorganisms, significantly improving the turnaround time for pathogen identification compared to traditional methods.[1,3] Our MALDI-TOF results largely corroborated the phenotypic identifications from Candichrome agar, while providing a more precise breakdown of species distribution across different sample types.

Consistent with the overall prevalence, *Candida albicans* was the dominant species in sputum (81.81%) and ETA (60%) samples, reinforcing its role as a significant colonizer and pathogen in the respiratory tract [9]. Its 100% presence in the single CSF isolate is clinically relevant, indicating its potential to cause severe central nervous system infections. In urine samples, *C. albicans* was still the most common (46.66%), but *C. tropicalis* (33.33%) and *C. glabrata* (13.33%) also contributed significantly, reflecting the varied etiology of candiduria [41, 42].

The distribution in blood samples is particularly important given the severity of candidemia. Here, *C. albicans* (54.54%) and *C. tropicalis* (36.36%) were the primary isolates. While *C. albicans* is traditionally the leading cause of candidemia, the high proportion of *C. tropicalis* aligns with global trends showing an increasing incidence of *C. tropicalis* candidemia, which is often associated with higher mortality rates in some settings[1,2,5]. The detection of *Candida krusei* (9.09%) in blood, though only one isolate, is notable because this species is intrinsically resistant to fluconazole, a commonly used antifungal[13,17]. Similarly, *C. parapsilosis* in pus samples highlights its potential role in wound infections and device-related infections [3,10].

This study underscores the critical need for species-level identification in managing candidiasis, especially given the varying antifungal susceptibility profiles of NAC species. Timely identification supports appropriate antifungal therapy and resistance prevention. These observations are in line with recommendations by Tajaneet al.[4] and Tiwari & Verma,[6] who advocate for integrated diagnostics combining phenotypic and molecular tools for effective fungal management.

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The findings from our study are crucial for developing effective antifungal stewardship programs and improving patient outcomes in our tertiary care hospital. The prevalence of *C. albicans* reaffirms the need for continued surveillance and appropriate treatment protocols for this species. However, the significant presence of NAC species, particularly *C. tropicalis*, necessitates vigilant identification and antifungal susceptibility testing to guide targeted therapy, especially considering their varying susceptibility profiles.[6,13].

The application of advanced diagnostic techniques like MALDI-TOF MS in routine laboratory practice provides rapid and accurate identification, facilitating timely intervention and reducing the reliance on empirical antifungal therapy[1,3]. This is particularly important in managing critically ill patients with underlying risk factors such as diabetes mellitus, immunosuppression (e.g., HIV, organ transplantation), and malignancy, who are highly susceptible to invasive candidiasis [26, 27, 29, 30, 33, 36, 38, 39, 40]. Furthermore, the emerging threat of multidrug-resistant *Candida* species, such as *Candida auris*, though not detected in our study, emphasizes the need for ongoing surveillance and robust infection control measures[15,18,25].

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Summary

This cross-sectional study was conducted over a four-month period at Dr. S.N. Medical College, Jodhpur, with the aim of investigating the phenotypic identification and species distribution of *Candida* isolates from various clinical samples.

Out of 400 processed samples, 74 (18.5%) tested positive for *Candida*, with the most frequent isolates found in sputum and urine samples. The use of both conventional culture methods and advanced techniques like MALDI-TOF MS allowed accurate species-level identification.

A total of 74 Candida isolates were identified. Candida albicans was the predominant species, accounting for 46 (62.16%) isolates, followed by Candida tropicalis with 22 (29.72%) isolates. Less frequently isolated species included Candida kefyr and Candida glabrata (2.70% each), while Candida parapsilosis and Candida krusei accounted for 1.35% each.

This reflects an epidemiological transition from *C. albicans* dominance to the rise of NAC species, emphasizing the need for precise diagnostic tools. The gender distribution showed a slight male predominance (55.41%), while urban cases slightly outnumbered rural ones (58.11% vs. 41.89%), suggesting more frequent healthcare access or reporting in urban regions.

Age-wise, children aged 0–10 years (33.78%) and the elderly population above 60 years (27.02%) were most affected, indicating bimodal vulnerability due to immune immaturity and immunosenescence, respectively. The study also found a much higher infection rate among hospitalized patients (71.62%) compared to outpatients, underlining the risk factors linked to hospital settings such as catheterization, ICU stays, and immunosuppression.

The application of MALDI-TOF MS significantly improved the speed and accuracy of species-level identification, especially for NAC isolates. This reinforces the importance of integrating advanced diagnostic modalities alongside traditional phenotypic methods to facilitate prompt and appropriate antifungal treatment, particularly in tertiary care setups.

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Conclusion

This study provides valuable insights into the epidemiological profile of *Candida* infections in a tertiary care setting. The data underscores the continued dominance of *C. albicans* while highlighting the significant contribution of NAC species, particularly *C. tropicalis*, across various clinical samples. The use of advanced identification methods like MALDI-TOF is indispensable for accurate and timely diagnosis, which is paramount for effective antifungal management and combating the evolving challenge of candidiasis in healthcare. Continued surveillance and research are vital to monitor changes in *Candida* epidemiology and antifungal resistance patterns to inform clinical guidelines and improve patient care.

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Limitation of study

In this present study only identification of Candida isolates was done. Antifungal susceptibility test for Candida isolates was not done.

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