INTERNATIONAL JOURNAL FOR INNOVATIVE RESEARCH IN MULTIDISCIPLINARY FIELD
ISSN(O): 2455-0620 [Impact Factor: 9.47]
Monthly, Peer-Reviewed, Refereed, Indexed Journal with IC Value : 86.87
Volume - 11, Issue - 05, May - 2025

DOIs:10.2015/IJIRMF/202505005 --:-- Research Paper / Article / Review

Typing of *Candida albicans* in UTI and Oral Swabs Using Gel Electrophoresis and Antifungal Assays

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Abstract : *This abstract is a summary of a study that examined Candida albicans strains isolated from Urinary Tract Infections (UTIs) and Oral Candidiasis to determine their molecular epidemiology, phenotypic features, and antifungal susceptibility. Candida albicans is an important opportunistic fungal pathogen, and the frequency of infections, such as UTIs and oral candidiasis, and antifungal resistance presents a clinical problem. This research utilized Molecular Typing based on Random Amplified Polymorphic DNA-PCR (RAPD-PCR) and Gel Electrophoresis for differential strain and Genotype identification. Phenotypic traits, such as Antifungal Susceptibility Testing in response to ordinary antifungals such as Fluconazole, were determined as well. The findings exhibited high genetic divergence among C. albicans from various body localizations, whereby different genotypes were correlated with UTIs as well as infections of the mouth. A significant observation was the identification of Fluconazole Resistance in a portion of isolates, which indicates possible treatment implications. Specific genotypes and resistance patterns were correlated, indicating dissemination of resistant clones. The present work adds to the knowledge of the Epidemiology and strain dynamics of C. albicans infections and emphasizes the significance of molecular typing and susceptibility testing for effective control and management of candidiasis.*

Keywords: *Candida albicans; Urinary Tract Infection (UTI); Oral Candidiasis; Molecular Typing; RAPD-PCR; Gel Electrophoresis; Antifungal Susceptibility Testing; Fluconazole Resistance; Strain Differentiation; Genotype; Phenotype; Epidemiology.*

1. INTRODUCTION:

Candida species, particularly *C. albicans*, are common commensals of the human microbiota that can become opportunistic pathogens(Neville et al., 2015). This shift is influenced by host (immune status, disease, age), microbial (virulence, biofilm, morphogenesis), and external factors (antibiotics, devices, hospitalization). *C. albicans*'s dimorphism and phenotypic switching contribute to its prevalence((Deng et al., 2021).

C. albicans causes a spectrum of infections, from superficial (oral thrush, vulvovaginal) to deep-seated and candidemia. Oral candidiasis is prevalent in specific populations and manifests in various forms. Its pathogenesis involves adhesion, invasion, biofilm formation, and host response((Asawari et al., 2025). Candida urinary tract infections are common nosocomial infections linked to catheters and other factors, ranging from asymptomatic candiduria to severe conditions. Diagnosis and management are challenging due to the need to differentiate colonization from true infection((Fisher et al., 2011).

Molecular typing methods have superseded traditional biotyping due to higher discrimination and reproducibility. Gel electrophoresis-based methods like RFLP and PFGE offer good discrimination but have limitations(Oakley et al., 2015). RAPD-PCR is simpler but less reproducible. Sequence-based methods like MLST and WGS provide higher resolution and are considered gold standards. The choice of method depends on the study's goals and resources(Ranjbar et al., 2014).

Antifungal susceptibility testing (AST) is vital for guiding therapy and monitoring resistance. Major antifungal classes include polyenes, azoles (with increasing resistance via target modification and efflux pumps), and echinocandins (resistance primarily through *FKS* mutations)(Lamoth et al., 2021). Standardized AST methods (CLSI, EUCAST) and

clinical breakpoints are essential for interpretation. Global trends show increasing azole resistance in *C. albicans* (Arendrup et al., 2024).

This study aims to address the limited local data comparing genotypes and phenotypes of *C. albicans* from urinary and oral sources. Its objectives are to isolate and identify *C. albicans*, investigate their genetic relatedness using RAPD-PCR, determine their antifungal susceptibility profiles, and explore potential associations between genotypes, susceptibility patterns, and anatomical source. The study proposes hypotheses regarding genetic diversity, site-specific clusters, resistance variations, and genotype-phenotype correlations.

2. MATERIAL METHODS :

2.1. Study Design and Ethical Considerations

- Study type: Prospective cross-sectional study.
- Study period:3.01.2025 to 4.4.2025
- Study location(s): Departments of Biochemistry at Kalinga University, Raipur, Chhattisgarh.
- Informed consent: Description of how patient consent was obtained. Patient confidentiality measures.

2.2.Patient Population and Sample Collection

- Inclusion criteria: Patients presenting with clinical signs suggestive of oral candidiasis (e.g., white plaques, erythema) or UTI (dysuria, frequency, urgency, positive urine culture defined by institutional criteria); age range [>18 years]. *C. albicans* isolated from the relevant sample.
- Exclusion criteria: Patients unable to provide consent; polymicrobial infections where *Candida* role is unclear; samples improperly collected or transported.
- Oral Swab Collection: Detailed procedure - site(s) swabbed (buccal mucosa, tongue dorsum, palate, denture surface), type of sterile swab used (cotton, Dacron), placed into transport medium (Amies, Stuart's, or sterile saline), immediate transport to the laboratory at room temperature/refrigerated.
- Urine Sample Collection: Detailed procedure - Midstream clean-catch urine (instructions given to patients), or sterile collection from indwelling urinary catheters (specify technique, aspiration from sampling port after clamping). Volume collected (10-20 mL). Transport conditions (sterile container, refrigeration if delay >2 hours, maximum transport time).

2.3. Isolation and Identification of *Candida albicans*

- Sample processing upon lab arrival: Urine centrifugation (speed, time) or direct plating of calibrated loop volume (1 µL, 10 µL). Oral swab plating (direct streaking).
- Culture Media: Primary isolation on Sabouraud Dextrose Agar (SDA) (Manufacturer, Cat. No.) supplemented with antibiotics (chloramphenicol, gentamicin) to inhibit bacterial growth. Incubation at [30°C or 35-37°C] for [24-72 hours]. Observation for yeast colonies(Smithee et al., 2014).
- Presumptive Identification: Colony morphology on SDA (creamy, white colonies), Gram staining (Gram-positive oval budding yeast cells).
- Species Identification:
  - CHROMagar Candida (Manufacturer, Cat. No.): Incubation [Temp, Time]. Observation for characteristic green colonies indicative of *C. albicans*(Pincus et al., 2007).
  - Germ Tube Test: Inoculation into serum (human, fetal bovine) and incubation at 37°C for 2-3 hours. Microscopic examination for germ tube formation(Makwana et al., 2012).
  - Confirmatory Identification (if needed or for all): Biochemical methods (API 20C AUX, Vitek 2 YST card - BioMérieux; specify system and procedure) OR Molecular confirmation (PCR targeting ITS region or species-specific *C. albicans* gene like HWP1). Detail PCR primers, conditions if used(Książczyk et al., 2016).
- Purity Check: Subculturing single colonies onto fresh SDA plates.
- Stocking Isolates: Long-term storage method (in glycerol broth [percentage] at -80°C or sterile water at room temp/4°C). Assign unique identifiers to each isolate.

2.4. DNA Extraction

- Culture Preparation: Grow isolate from stock on SDA [Temp, Time]. Harvest fresh yeast cells.
- Extraction Method (Choose ONE and detail extensively, or describe the kit used):
  - *Example: Enzymatic/Mechanical Lysis Method:* Cell washing steps (e.g., sterile water, PBS). Resuspension in lysis buffer (composition: e.g., Tris-HCl, EDTA, Sorbitol). Enzymatic treatment (e.g., Lyticase or Zymolyase - concentration, incubation time/temp). Mechanical disruption (e.g., bead beating with glass beads - specify bead size, vortexer type, time, cycles). Proteinase K treatment (concentration, incubation time/temp). DNA purification steps (e.g., Phenol: Chloroform: Isoamyl alcohol extraction, followed by ethanol precipitation; OR using silica-based columns from a commercial kit - specify kit name, manufacturer, Cat. No., and protocol steps followed). DNA elution (elution buffer, volume).
- DNA Quality and Quantity Assessment:

Spectrophotometry: Nanodrop (Thermo Fisher Scientific) or similar. Measure absorbance at 260 nm (DNA) and 280 nm (protein). Calculate concentration (ng/µL) and A260/A280 ratio (aim for ~1.8-2.0 for purity). Measure A260/A230 ratio (aim for >1.8 to check for salt/solvent contamination)(Rodríguez-Riveiro et al., 2022)
Agarose Gel Electrophoresis: Run a small aliquot (e.g., 50-100 ng) on a [e.g., 0.8%] agarose gel alongside a known DNA ladder to check for integrity (high molecular weight band, minimal shearing/degradation) and confirm concentration roughly.

- DNA Storage: Store extracted DNA at [-20°C or -80°C] until use.

2.5. Molecular Typing by RAPD-PCR

- Primer Selection: Justify primer choice (previously published, shown good discrimination for *C. albicans*). Specify primer sequence(s) (Primer OPE-18:5'-GGACTGCAGA-3'; Primer ERIC2:5'-AAGTAAGTGACTGGGGTGAGCG-3'). Source of primers (synthesis company). Reconstitution and working concentration (e10 pmol/µL).
- PCR Reaction Mixture: Detail exact components per reaction (total volume 25 µL):Template DNA: [Amount, 25-50 ng], Primer: [Concentration, 0.5 µM or 10-20 pmol], dNTP Mix: [Final concentration of each dNTP, 200 µM], MgCl2: [Final concentration, 1.5 - 3.0 mM - optimization might be needed], Taq DNA Polymerase: [Units per reaction, 1-1.25 U] (Specify type/brand, Invitrogen, Promega), PCR Buffer: [Concentration, e1X] (Supplied with enzyme),Nuclease-free water: To final volume(Asif et al., 2021)
- PCR Amplification Conditions: Specify the thermal cycler used (Brand, Model). Detail the cycling program:
  - Initial Denaturation: [94°C for 5 min],Cycles (40 cycles):Denaturation: [94°C for 1 min], Annealing: e.g., 36°C for 1 min , Extension: [72°C for 2 min],Final Extension: [72°C for 10 min],Hold: [4°C](Walsh et al., 1992)
- Controls:
  - Positive Control: Include DNA from a reference *C. albicans* strain (e.g., ATCC 90028) in each run(Ramage et al., 2001)
  - Negative Control: Replace template DNA with nuclease-free water to check for contamination(Zozaya-Valdés et al., 2021).
- Reproducibility Check: Run select isolates in duplicate or triplicate on different days to ensure pattern consistency.

2.6. Agarose Gel Electrophoresis of RAPD Products

- Gel Preparation: [Concentration, e.g., 1.5%] agarose (Brand) in [Buffer, e.g., 1X TBE or 0.5X TBE]. Include DNA stain (e.g., Ethidium Bromide [final conc. 0.5 µg/mL] added to gel OR post-staining; alternatively SYBR Safe - specify). Gel casting tray and comb size (14)(Dash et al., 2016)
- Sample Loading: Mix 10 µL of PCR product with volume of 6X loading dye (Composition: bromophenol blue, xylene cyanol, glycerol). Load into wells(Menossi et al., 2000)

- Molecular Weight Marker: Load a suitable DNA ladder).
- Electrophoresis Conditions: Run gel submerged in the same buffer used to make the gel. Specify voltage [80-100 V] and run time [2-3 hours or until dye front migrates sufficiently]. Electrophoresis tank .
- Visualization and Documentation: Visualize DNA bands under UV light using a UV transilluminator. Capture digital images using a gel documentation system. Ensure image quality is suitable for analysis.

2.7. Antifungal Susceptibility Testing (AST)

- Antifungal Agents Tested: List drugs (e.g., Fluconazole, Amphotericin B, Voriconazole, Caspofungin, Itraconazole, Micafungin). Source (e.g., Sigma-Aldrich, USP reference standards, commercial powders). Solvent used for stock solutions (e.g., DMSO, water). Stock concentration and storage.
- Inoculum Preparation:
  - Grow isolate on SDA [37 degree for 16-18 hours].
  - Suspend colonies in sterile saline (0.85%).
  - Adjust turbidity spectrophotometrically at 530 nm to match a 0.5 McFarland standard OR using a nephelometer. This corresponds to approx. 1-5 x 10^6 CFU/mL.
  - Dilute this suspension in RPMI medium to achieve the final working inoculum concentration. Verification by quantitative plating may be described.
- Microdilution Plate Preparation: Use sterile 96-well flat-bottom microtiter plates. Prepare serial two-fold dilutions of each antifungal drug in RPMI medium directly in the plates. Final drug concentration range tested for each drug (Fluconazole: 0.125 - 64 µg/mL; Amphotericin B: 0.03 - 16 µg/mL; Caspofungin: 0.015 - 8 µg/mL). Final volume per well [100 µL drug dilution + 100 µL inoculum](Cardoso et al., 2022).
- Controls in Each Plate:
  - Growth Control (GC): Drug-free well with inoculum.
  - Sterility Control (SC): Drug-free well with medium only.
- Incubation: Incubate plates at 35°C for [e.g., 24 hours for echinocandins and polyenes, 48 hours for azoles, or read both times]. Ambient air, static incubation(Van Dijck et al., 2018).
- Reading MICs (Minimum Inhibitory Concentration):
  - Determine visually or using a microplate reader (specify wavelength).
  - Endpoint definition (Crucial):

Azoles (Fluconazole, Voriconazole, Itraconazole): Concentration inhibiting ~50% of growth compared to the GC (CLSI M27).
Amphotericin B: Lowest concentration showing complete inhibition (optically clear).
Echinocandins (Caspofungin): Lowest concentration leading to significant diminution of growth (e.g., small, fuzzy colonies or slight haze) compared to the GC (CLSI M27) - note paradoxical growth effect possibility(Tóth et al., 2020).

- Quality Control (QC): Test reference strains with known MIC ranges in parallel with each batch of tests (e.g., *C. parapsilosis* ATCC 22019, *C. krusei* ATCC 6258). Ensure obtained MICs fall within published CLSI acceptable ranges(Arendrup et al., 2014).

2.8. Data Analysis

- RAPD Pattern Analysis:
  - Image Processing: Use gel analysis software [BioNumerics (Applied Maths), GelCompar II (Applied Maths), PyElph, GelJ, or similar]. Normalize lanes using the molecular weight marker.

- Band Scoring: Manually or automatically identify clear, reproducible bands within a defined molecular weight range (e.g., 200 bp - 3000 bp). Create a binary matrix (1=presence, 0=absence) for each band position across all isolates. Faint or ambiguous bands should be excluded.

- Cluster Analysis: Construct a dendrogram using an agglomerative clustering algorithm [UPGMA (Unweighted Pair Group Method with Arithmetic Mean)].
- Genotype Definition: Define distinct genotypes based on a similarity cutoff value [≥80% or ≥90% similarity considered the same genotype/cluster]. Justify the chosen cutoff based on reproducibility tests or literature. Calculate discriminatory power using Simpson's Index of Diversity(Perreault et al., 2014)

2.9. Antifungal Susceptibility Data Analysis:

- Tabulate the number and percentage of isolates categorized as S, SDD/I, and R for each drug based on interpretive criteria.
- Identify multidrug-resistant (MDR) isolates if applicable (define MDR criteria, e.g., resistant to ≥2 drug classes).
- Antifungal used - FLC – Fluconazole, PB – Polymyxin B, AP – Amphotericin B, NA- – Nystatin, TR – Terbinafine, CLR – Clotrimazole, TEI – Teicoplanin, NIT – Nitrofurantoin ,KT – Ketoconazole, ITR – Itraconazole

3. RESULTS

3.1. Study Population and Isolate Collection

- Description of the cohort: Number of patients screened, number included. Basic demographics (age range, gender distribution) if available and ethical to report.
- Number of samples collected: Total oral swabs, total urine samples.
- Number of *C. albicans* isolates obtained: [N] isolates confirmed as *C. albicans*. [N_oral] from oral swabs, [N uti] from urine. Mention if multiple isolates were obtained from single patients.
- Table 1: Summary of Isolates (Isolate ID, Patient ID, Source, Date collected, [: Ward/Clinic]).

Isolate ID	Patient ID	source	Date collected	Word/ clinic
001	1243	UTI	12.01.2025	WORD-1
002	1244	UTI	20.01.2025	WORD-10
003	1245	UTI	28.01.2025	WORD-3
004	1246	UTI	4.02.2025	WORD-10
005	1247	UTI	15.02.2025	WORD-12

3.2. Molecular Typing by RAPD-PCR

- Amplification success rate: Mention if all isolates yielded amplifiable DNA and reproducible RAPD patterns.
- Description of RAPD patterns: Complexity (number of bands per isolate, e.g., ranging from 5 to 15 bands), size range of amplified fragments (200 bp to 3 kb).
- Figure 4.2.1 Representative RAPD gel image(s) showing patterns from different isolates (UTI and Oral), controls, and DNA ladder. Label lanes clearly.

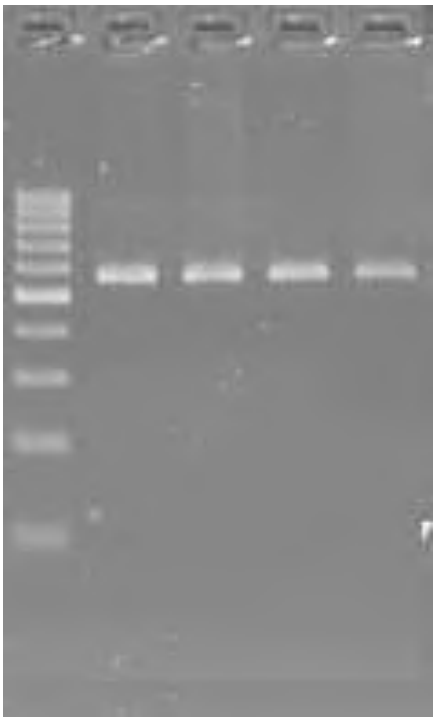

Fig-4.2.1

- Dendrogram Analysis:
  - Figure 4.2.2,4.2.3,4.2.4: Dendrogram showing the clustering of all [N] *C. albicans* isolates based on RAPD profiles (using Dice/UPGMA, include similarity scale). Mark clusters defined by the chosen similarity cutoff (80%). Indicate the source (Oral/UTI) for each isolate on the dendrogram (using different colors or symbols).
  - Genetic Diversity: Report the number of distinct RAPD genotypes (patterns) identified at the chosen cutoff. Calculate Simpson's Index of Diversity.
  - Clustering Patterns: Describe major clusters observed. Note the size of clusters (number of isolates). Mention any large clusters suggesting clonal expansion. Note singleton isolates (unique patterns).
  - Source Association: Analyze the distribution of UTI vs. Oral isolates within the dendrogram. Are there clusters predominantly or exclusively composed of isolates from one source. Report statistical association if tested (certain clusters significantly associated with UTI or Oral source).
  - Intra-patient relatedness (if applicable): If multiple isolates were typed from the same patient (oral and urine, or repeat samples), report if they were genetically identical or different based on RAPD.

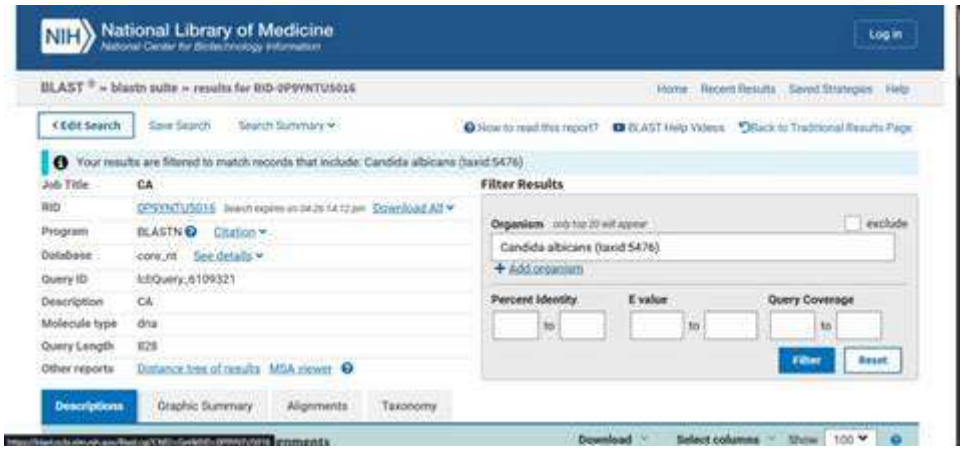

Fig-4.2.2

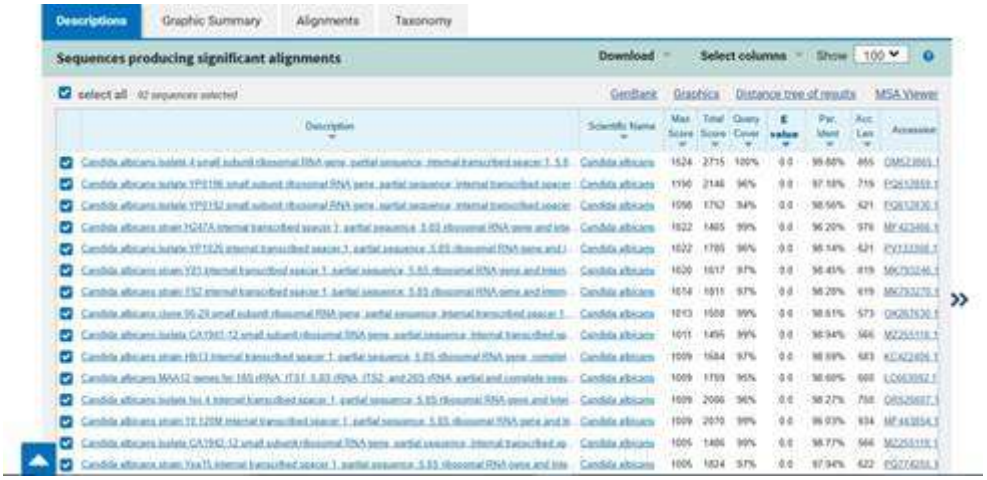

Fig-4.2.3

Fig-4.2.4

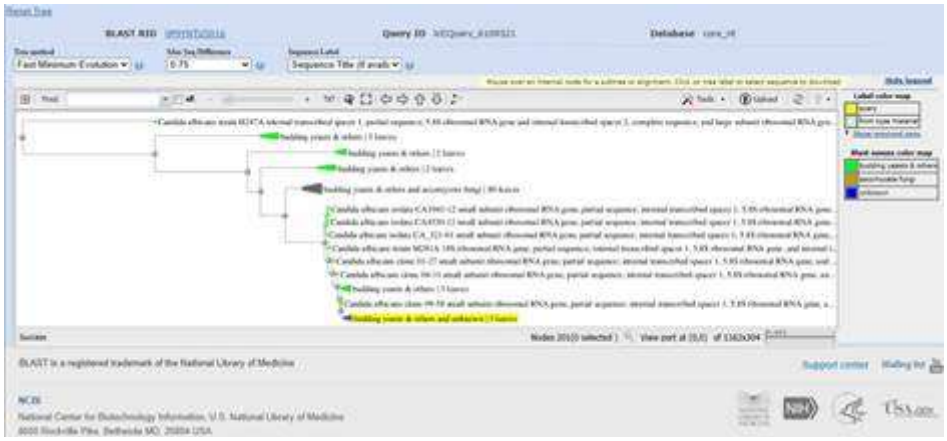

Fig-4.2.5

3.3. Antifungal Susceptibility Profiles

- QC Strain Results: State that MICs for QC strains were within acceptable ranges for all test runs.
- Resistance Rates:
  - Table 2: Number and percentage of isolates classified as Susceptible (S), Susceptible Dose-Dependent/Intermediate (SDD/I), and Resistant (R) for each antifungal, based on CLSI/EUCAST breakpoints. Present overall rates and rates stratified by source (UTI vs. Oral).

- Highlight key findings: e.g., Overall high susceptibility to Amphotericin B and Echinocandins. Specify resistance rate for Fluconazole, Voriconazole. Compare resistance rates between UTI and Oral isolates statistically (report p-values from Chi-squared/Fisher's test).
- Multidrug Resistance: Report the number/percentage of MDR isolates found and their resistance patterns.

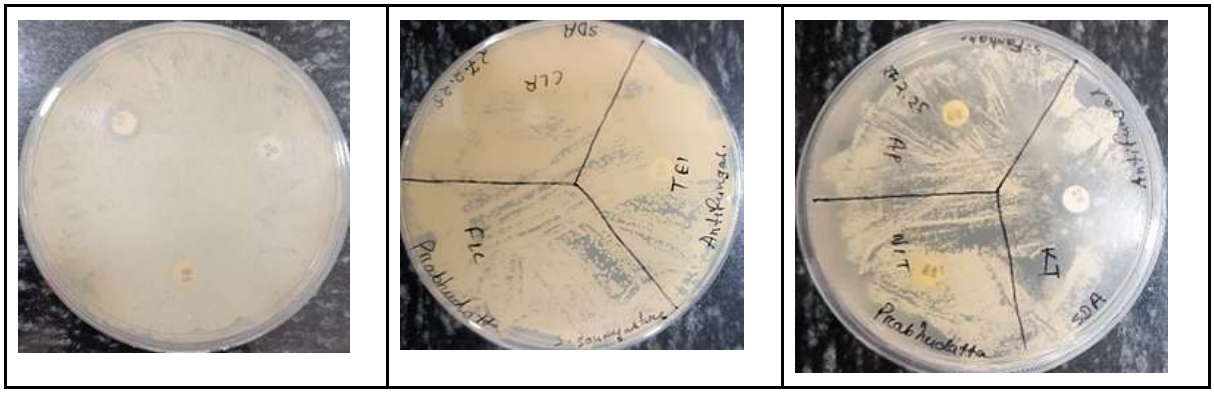

Fig- 4.3.1

4.DISCUSSION

4.1. Summary of Key Findings

- Briefly reiterate the main results regarding genetic diversity, clustering, antifungal susceptibility patterns, and correlations found.

4.2.Genetic Diversity and Population Structure of *C. albicans*

- Interpret the level of genetic diversity observed (high diversity suggests a heterogeneous population, low diversity might suggest dominant clones). Compare the calculated diversity index (Simpson's) with other studies using RAPD or other methods on *C. albicans* from similar/different sources or geographical locations.
- Discuss the implications of the observed diversity – does it reflect endogenous strain carriage variability, patient-to-patient transmission, or microevolution.
- Discuss the clustering observed. Do the clusters represent known clades of *C. albicans* (though RAPD may not resolve major clades well compared to MLST).
- Reliability of RAPD: Acknowledge the limitations of RAPD (reproducibility issues). Discuss steps taken to mitigate this (standardized protocol, controls, duplicate runs). State that findings should ideally be confirmed with more robust methods like MLST or WGS, but RAPD provided a useful initial screen in this context.

4.3. Comparison of Isolates from UTI and Oral Sources

- Discuss the findings regarding genetic relatedness between UTI and oral isolates. Were they largely distinct populations or overlapping?
- If overlap exists (isolates from both sources within the same cluster): Discuss potential implications – systemic carriage with manifestation at different sites, potential for translocation (gut reservoir seeding both oral cavity and urinary tract), contamination during sampling (less likely if procedures were strict).
- If distinct populations exist: Discuss potential niche adaptation – are certain genotypes better suited for survival/colonization/invasion in the urinary tract versus the oral cavity Link to known virulence factors if possible .
- Compare findings with literature comparing *C. albicans* from different body sites.

4.4. Antifungal Susceptibility Patterns and Resistance

- Discuss the observed susceptibility rates for each drug class.
- Focus on resistance findings: Discuss the clinical significance of the observed resistance rates.

- Compare resistance rates between UTI and Oral isolates. If differences were found), speculate on reasons
- Acknowledge limitations of in vitro AST predicting in vivo outcome.

4.5. Genotype-Phenotype Correlations

- Discuss the strength and significance of any observed correlations between specific RAPD genotypes and antifungal resistance. If found, does this suggest clonal spread of resistant strains?
- If no strong correlation was found, discuss why: resistance mechanisms might be acquired independently of genetic background), RAPD may not capture the relevant genetic variation linked to resistance, complex genotype-phenotype relationship.
- Discuss any correlation (or lack thereof) between genotype and source. Does it support the idea of niche-adapted strains?

4.6.Clinical and Epidemiological Implications

- How can these findings inform clinical practice?
- How can the typing information be used? (e.g., investigating suspected outbreaks, understanding if recurrent infections are relapse or reinfection).
- Implications for infection control, especially if common clones were found across multiple patients or linked to specific wards.

4.7. Strengths and Limitations of the Study

- Strengths: (e.g., simultaneous comparison of UTI and oral isolates from the same setting, use of standardized methods for AST, confirmation of species ID, inclusion of reasonable number of isolates).
- Limitations:
  - Methodological limitations (RAPD reproducibility and discriminatory power vs. MLST/WGS).
  - Study design limitations (cross-sectional design cannot infer causality or track changes over time; single-center study limits generalizability).
  - Sample size (might be underpowered for some statistical comparisons).
  - Lack of clinical data linkage (correlation with patient outcomes, treatment history, underlying conditions was not performed).
  - Lack of investigation into resistance mechanisms.
  - Potential bias in patient selection or sample collection.

5. Future Directions

- Suggestions for further research:
  - Confirm findings using more robust typing methods (MLST, WGS).
  - Investigate resistance mechanisms in resistant isolates (gene sequencing, expression analysis).
  - Correlate findings with detailed clinical data (patient demographics, comorbidities, antifungal exposure, treatment outcomes).
  - Longitudinal studies to track strain persistence/replacement over time within patients.
  - Expand study to multiple centers.
  - Investigate virulence factors (e.g., biofilm formation, enzyme production) and correlate with genotype/source.

6. CONCLUSION

- . This study revealed considerable genetic heterogeneity among *C. albicans* isolates causing UTIs and oral candidiasis in , as demonstrated by RAPD-PCR typing.
- Reiterate the main conclusions regarding comparison between sources (While some overlap exists, suggesting potential endogenous spread, hints of source-specific clustering warrant further investigation).

- Reiterate key findings on antifungal susceptibility (High susceptibility to AmB and echinocandins persists, but azole resistance, particularly to fluconazole, was observed at a rate of 10% and requires ongoing monitoring, possibly differing slightly between UTI and oral isolates).
- State the overall significance: The combined use of molecular typing, even with simpler methods like RAPD, and standardized antifungal susceptibility testing provides valuable data for understanding local *C. albicans* epidemiology, potential transmission dynamics, and guiding appropriate therapeutic management. Further studies using higher-resolution methods and incorporating clinical data are needed to build upon these findings.

REFERENCES:

1. Neville, B. A., d'Enfert, C., & Bougnoux, M. E. (2015). Candida albicans commensalism in the gastrointestinal tract. *FEMS yeast research*, *15*(7), fov081.
2. Deng, Y., Li, S., Bing, J., Liao, W., & Tao, L. (2021). Phenotypic switching and filamentation in Candida haemulonii, an emerging opportunistic pathogen of humans. *Microbiology Spectrum*, *9*(3), e00779-21.
3. Asawari, P., Talreja, S., Rohan, B., & Vijay, J. (2025). Unraveling Candidiasis: Pathogenic Mechanisms and the Spectrum of Infections. *Asian Journal of Pharmaceutical Research and Development*, *13*(1), 174-180.
4. Fisher, J. F., Kavanagh, K., Sobel, J. D., Kauffman, C. A., & Newman, C. A. (2011). Candida urinary tract infection: pathogenesis. *Clinical Infectious Diseases*, *52*(suppl_6), S437-S451.
5. Oakley, B. B., Gonzalez-Escalona, N., & Molina, M. (2015). Molecular typing and differentiation. *Compendium Methods Microbiological Exams in Foods.*, 72-153.
6. Ranjbar, R., Karami, A., Farshad, S., Giammanco, G. M., & Mammina, C. (2014). Typing methods used in the molecular epidemiology of microbial pathogens: a how-to guide. *New Microbiologica*, *37*(1), 1-15.
7. Lamoth, F., Lewis, R. E., & Kontoyiannis, D. P. (2021). Role and interpretation of antifungal susceptibility testing for the management of invasive fungal infections. *Journal of Fungi*, *7*(1), 17.
8. Smithee, S., Tracy, S., Drescher, K. M., Pitz, L. A., & McDonald, T. (2014). A novel, broadly applicable approach to isolation of fungi in diverse growth media. *Journal of Microbiological Methods*, *105*, 155-161.
9. Pincus, D. H., Orenga, S., & Chatellier, S. (2007). Yeast identification—past, present, and future methods. *Medical mycology*, *45*(2), 97-121.
10. Makwana, G. E., Gadhavi, H., & Sinha, M. (2012). Comparison of germ tube production by Candida albicans in various media. *NJIRM*, *3*, 6-8.
11. Rodríguez-Riveiro, R., Velasco, A., & Sotelo, C. G. (2022). The influence of DNA extraction methods on species identification results of seafood products. *Foods*, *11*(12), 1739.
12. Asif, S., Khan, M., Arshad, M. W., & Shabbir, M. I. (2021). PCR optimization for beginners: a step by step guide. *Research in Molecular Medicine*, *9*(2), 81-102.
13. Asif, S., Khan, M., Arshad, M. W., & Shabbir, M. I. (2021). PCR optimization for beginners: a step by step guide. *Research in Molecular Medicine*, *9*(2), 81-102.
14. Walsh, P. S., Erlich, H. A., & Higuchi, R. (1992). Preferential PCR amplification of alleles: mechanisms and solutions. *Genome Research*, *1*(4), 241-250.
15. Ramage, G., Vande Walle, K., Wickes, B. L., & López-Ribot, J. L. (2001). Standardized method for in vitro antifungal susceptibility testing of Candida albicans biofilms. *Antimicrobial agents and chemotherapy*, *45*(9), 2475-2479.
16. Zozaya-Valdés, E., Wong, S. Q., Raleigh, J., Hatzimihalis, A., Ftouni, S., Papenfuss, A. T., ... & Dawson, S. J. (2021). Detection of cell-free microbial DNA using a contaminant-controlled analysis framework. *Genome Biology*, *22*(1), 187.
17. Dash, P., Rai, R., Padaria, J. C., & Rohini, S. (2016). Practical Manual: Winter School on Marker free transgenics and methods of transgene detection sponsored by ICAR; 1-21 February, 2016. *ICAR-National Research Centre on Plant Biotechnology, New Delhi, India*, *125*.
18. Menossi, M., Cremonese Jr, N., Maron, L. G., & Arruda, P. (2000). Making colony PCR easier by adding gel-loading buffer to the amplification reaction. *BioTechniques*, *28*(3), 424-426.
19. Cardoso, J., Freitas-Silva, J., Durães, F., Carvalho, D. T., Gales, L., Pinto, M., ... & Pinto, E. (2022). Antifungal Activity of a Library of Aminothioxanthones. *Antibiotics*, *11*(11), 1488.
20. Van Dijck, P., Sjollema, J., Cammue, B. P., Lagrou, K., Berman, J., d'Enfert, C., ... & Thevissen, K. (2018). Methodologies for in vitro and in vivo evaluation of efficacy of antifungal and antibiofilm agents and surface coatings against fungal biofilms. *Microbial Cell*, *5*(7), 300.

21. Tóth, Z., Forgács, L., Kardos, T., Kovács, R., Locke, J. B., Kardos, G., ... & Majoros, L. (2020). Relative frequency of paradoxical growth and trailing effect with caspofungin, micafungin, anidulafungin, and the novel echinocandin rezafungin against Candida species. *Journal of Fungi*, *6*(3), 136.
22. Arendrup, M. C., Howard, S., Lass-Flörl, C., Mouton, J. W., Meletiadis, J., & Cuenca-Estrella, M. (2014). EUCAST testing of isavuconazole susceptibility in Aspergillus: comparison of results for inoculum standardization using conidium counting versus optical density. *Antimicrobial Agents and Chemotherapy*, *58*(11), 6432-6436.
23. Perreault, L. P. L., Legault, M. A., Barhdadi, A., Provost, S., Normand, V., Tardif, J. C., & Dubé, M. P. (2014). Comparison of genotype clustering tools with rare variants. *BMC bioinformatics*, *15*, 1-12.