International Journal of Life Sciences, Biotechnology and Pharma Research Vol. 15, No. 3, March 2026 Online ISSN: 2250-3137
Print ISSN: 2977-0122

DOI: 10.69605/ijlbpr_15.3.2026.74

**ORIGINAL RESEARCH**

# Speciation of Candida using Conventional Method and CHROMagar Isolated from Various Clinical Specimens

[1]Miss Neeraj Kumari, [2]Dr. Madhurendra Singh Rajput

[1]Ph.D scholars (Medical Microbiology), [2]Professor, Department of Microbiology, Malwanchal University, Indore, Madhya Pradesh, India

**Corresponding author**
Miss Neeraj Kumari
Ph.D scholars (Medical Microbiology), Department of Microbiology, Malwanchal University, Indore, Madhya Pradesh, India
**Email:** nimmy89.du@gmail.com

Received: 03 February, 2026 Acceptance: 06 March, 2026 Published: 11 March, 2026

## ABSTRACT

**Background:** Candida spp are part of the normal microbial flora found on the skin, mucous membranes, and within the gastrointestinal tract. *Candida* species are the leading opportunistic fungal pathogens responsible for severe infections, particularly in individuals who are immunocompromised, elderly, receiving prolonged antibacterial therapy or intensive chemotherapy, or undergoing invasive surgical procedures and organ transplantation. The present study was conducted to study the distribution of Candida albicans and non albicans Candida from various clinical samples and to speciate Candida using CHROMagar Candida differential agar. **Material and Methods:** This prospective cross-sectional study was conducted in the Mycology Laboratory, Department of Microbiology. The preliminary diagnosis was made by Gram stain, KOH mount, Culture on Sabouraud's dextrose agar and CHROMagar Candida differential agar were used for speciation of Candida. **Results:** A total of 250 *Candida* species isolates were identified, with an overall prevalence of 3.27%. The majority of the isolates were obtained from female patients (52.4%), while 47.6% were from male patients. The age group most commonly affected by candidiasis was 21–40 years (39.2%), followed by 41–50 years (28.8%). Candida albicans was the most frequently isolated species (57.6%), followed by Candida tropicalis (37.6%), Candida glabrata (2.8%), and Candida parapsilosis (2%) respectively. **Conclusion:** *Candida* species are opportunistic pathogenic organisms. CHROMagar is a simple, rapid, and cost-effective method for the identification of *Candida* species, even in resource-limited settings.

**Keywords:** Candida albicans, Non-albicans Candida, CHROMagar and Conventional method.

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-Share Alike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

## INTRODUCTION

*Candida* species are important opportunistic fungal pathogens responsible for a wide range of human infections[1]. *Candida* species are natural components of the normal human microbiota and commonly inhabit the skin, oral cavity, gastrointestinal tract, and genitourinary mucosa[2]. They are endogenous opportunistic organisms that can cause secondary infections in individuals with underlying immunocompromised conditions, such as acquired immunodeficiency syndrome, pregnancy, those undergoing surgery or organ transplantation, and patients receiving chemotherapy[3,4].

Candidiasis can be classified into three main categories: cutaneous candidiasis, which involves the skin and its appendages; mucosal candidiasis, which affects the oropharynx, esophagus, and vulvovaginal region; and systemic candidiasis, which involves the bloodstream (candidemia) and other forms of invasive infection.[5]

Invasive candidiasis is a life-threatening infection caused by different *Candida* species. Although Candida albicans has traditionally been the predominant cause of invasive candidiasis, non-albicans *Candida* (NAC) species have increasingly emerged worldwide as significant pathogens[6]. In India, Candida tropicalis is the most frequently isolated *Candida* species from blood samples[7].

There are currently over 150 identified *Candida* species, but only about 20 of these are known to be pathogenic in humans[8]. While Candida albicans has traditionally been regarded as the primary pathogen responsible for candidemia, there has been a noticeable shift toward infections caused by non-albicans *Candida* species [9].

Conventional diagnostic methods such as Gram staining of smears, the germ tube test, colony morphology on Sabouraud's dextrose agar (SDA), the

DOI: 10.69605/ijlbpr_15.3.2026.74

urease test, growth on corn meal agar, sugar fermentation and assimilation tests, and growth at 45 °C—are labour intensive, cumbersome, and time-consuming[10]. Modern techniques developed for yeast identification include CHROMagar, API systems, the VITEK 2 ID system, and molecular methods[11].

Therefore, accurate identification and speciation of *Candida* species are crucial for effective clinical management. Accordingly, the present study was designed to isolate *Candida* species from clinical specimens and determine their specific species using a range of phenotypic methods.

## MATERIAL AND METHODS

### Sample collection

The present study was carried out in the Department of Microbiology at index medical college hospital and research centre during the 2024-2025, Indore. A total of 250 *Candida* isolates were obtained from various clinical specimens, including urine, blood, high vaginal swabs (HVS), pus, wound, and skin swabs from patients. All samples were collected following strict aseptic precautions.

### Mycological examination Direct examination

These specimens were processed for the isolation of *Candida* species using standard mycological techniques. All clinical samples were initially screened for budding yeast- like cells through direct microscopy using Gram staining and 10% KOH preparations, as well as Gram staining of colonies grown on routine culture media[3] (Fig-1).

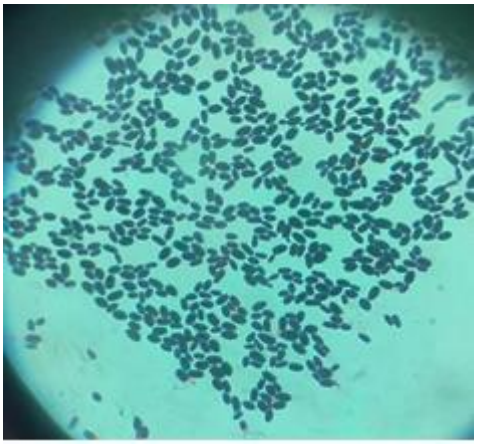


**Fig-1: Gram stain showing Candida.**

### Identification of Candida spp

The specimens were inoculated onto Blood Agar and Sabouraud's Dextrose Agar and incubated at 37 °C for 24–72 hours. On Sabouraud's Dextrose Agar (SDA), *Candida* colonies typically appeared creamy, pasty, and yeast-like in texture[8] (Fig-2).



**Fig-2: Candida growth on Sabouraud Dextrose Agar (SDA) culture plate**

The germ tube test was carried out to differentiate among *Candida* species. Samples that produced a positive reaction were identified as either *C. albicans* or *C. dubliniensis*, effectively distinguishing these from other *Candida* species based on their ability to form germ tubes under the test conditions[2]. On Corn Meal Agar (CMA), *Candida* displays distinct morphological features, such as chlamydospores, blastospores, and hyphal formation[12] (Fig-3).



©2026 Int. J. Life Sci. Biotechnol. Pharma. Res

DOI: 10.69605/ijlbpr_15.3.2026.74

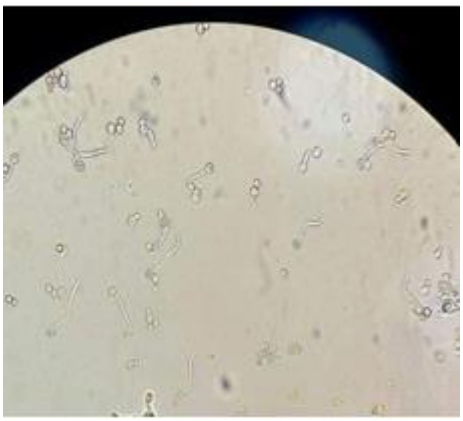


**Fig-3: Microscopic view of Germ tube formation**

**Colony Morphology of Candida spp**

The *Candida* isolates were cultured on CHROMagar differential medium and incubated at 37°C for 24 hours. Identification of the species was carried out by observing the distinctive colony morphology and pigmentation, in accordance with the manufacturer's guidelines[10]. (Fig-4)

**RESULT**

A total of 250 *Candida* isolates were obtained from a variety of clinical specimens. An overall prevalence of 10.19% for *Candida* species was observed in this study. Gender-wise distribution revealed that 52.4% of the *Candida* isolates were obtained from female patients, while 47.6% were from male patients, indicating a slightly higher prevalence among females **(Fig-5).** The age group most commonly affected by candidiasis was 21–40 years, accounting for 39.2% of cases, followed by the 41–60 years age group at 28.8%. The age- and gender-wise distribution of *Candida* isolates is presented in **Table-I**. *Candida* isolates were most frequently obtained from urine (33.6%) and blood (30%), with smaller proportions from HVS (14.4%), pus (12.8%), wound swabs (6.4%), and skin swabs (2.8%) showing in **Fig-6** and summarizes the species distribution among the various specimen. Among the total *Candida* isolates, *C. albicans* predominated, representing 57.6% of strains, whereas non- *albicans Candida* species comprised 42.4% of the isolates **(Table II).** Candida isolates were also subjected to identification using Hicrome Candida differential agar. For species- level identification, the *Candida* isolates were cultured on CHROMagar Candida differential agar, allowing differentiation based on colony colour and morphology. The identification of *Candida* species using both conventional methods and CHROMagar Candida differential agar revealed the following distribution: *C. albicans* (57.6%), *C. tropicalis* (37.6%), *C. glabrata* (2.8%), and *C. parapsilosis* (2%), as shown in **Table III and Fig-4.**

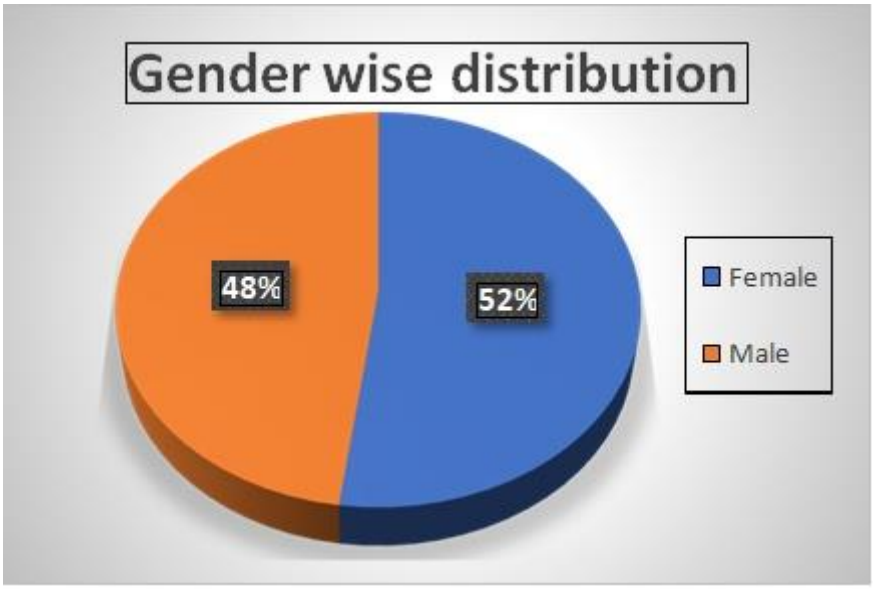



**Fig-5: Gender wise distribution of *Candida* isolates from various clinical samples**



©2026 Int. J. Life Sci. Biotechnol. Pharma. Res

DOI: 10.69605/ijlbpr_15.3.2026.74

**Table- I: Age Wise Distribution of Candida isolates from patient.**

| AGE GROUP | MALE (n=119) | FEMALE (n=131) | TOTAL (n=250) | PERCENTAG E (%) |
|---|---|---|---|---|
| 0-20 years | 4 | 5 | 09 | 3.60% |
| 21-40 years | 40 | 58 | 98 | 39.20% |
| 41-60 years | 40 | 32 | 72 | 28.80% |
| 61-80 years | 27 | 26 | 53 | 21.20% |
| >81 years | 8 | 5 | 13 | 5.20% |

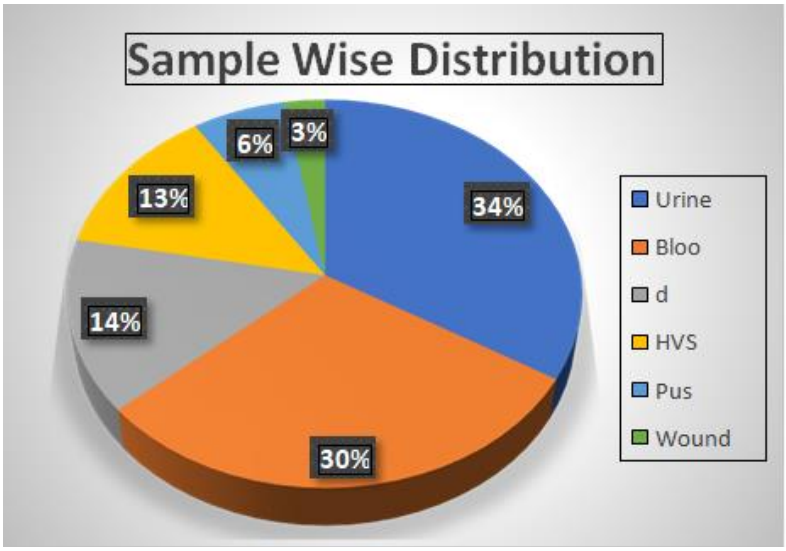



**Fig-6: Sample wise distribution of Candida isolates from patient**

**Table-II: Prevalence of Candida spp and Non-albicans Candida (NAC) spp.**

| Types of candida species | No. of isolates (n=250) | Percentage (%) |
|---|---|---|
| C. albicans | 144 | (57.60%) |
| Non-albicans Candida (NAC) | 106 | (42.40%) |

**Table-III: Identification of Candida species by CHROMagar Candida differential agar.**

| Species | No. of isolates (n=250) | Positive test |
|---|---|---|
| C. albicans | 144 (57.60%) | CHROMagar, green colonies |
| C. tropicalis | 94 (37.60%) | CHROMagar, blue colonies |
| C glabrata | 7 (2.80%) | CHROMagar, cream to light pink/purple colonies |
| C. parapsilosis | 5 (2.00%) | CHROMagar, white to cream colonies |

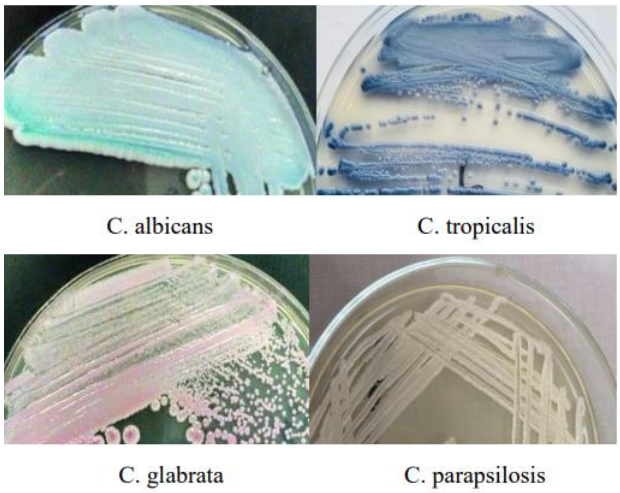



**Fig-4: Differentiation of various species of Candida on CHROMagar**.



©2026 Int. J. Life Sci. Biotechnol. Pharma. Res

DOI: 10.69605/ijlbpr_15.3.2026.74

## DISCUSSION

In the present study, the prevalence of Candida spp among the culture positive samples was found to the 10.19%. This observation aligns with the results of **Baniodeh H. et al. (2024),** where the prevalence of *Candida* species among clinical samples was reported to be 11.6%. These results are consistent with those reported by Solomon D.A. et al. (2021), where the prevalence of *Candida* species was found to be 8.2%. In another study conducted by **Al Halteet S, et al.,** (2020)[15] reported a higher prevalence of Candida species at 15.6%.

In the present study, *Candida* isolates were more frequently recovered from female patients (52.4%) compared to male patients (47.6%) (Fig-5). These results align with the study by Narayankar P. et al. (2025), which also observed a female predominance, with 52% of *Candida* infections occurring in female patients versus 48% in male patients. This is in agreement with the study by Aruna M. et al. (2025), which reported that 53.6% of *Candida* isolates were from female patients, while 46.4% were from male patients. Women of reproductive age are more prone to *Candida* infections, as hormonal changes and physiological conditions during this period create an environment conducive to yeast proliferation.

Among the age groups studied, the largest number of *Candida* isolates was found in participants aged 21–40 years (39.2%), followed by those aged 41–60 years (28.8%) **(TABLE-I)**. In a study conducted by Sachin B.K. et al. (2024), 41.25% of *Candida* isolates were obtained from patients in the 20–40 years age group. This finding is comparable to the study by Sugashini D. et al. (2025), in which the highest proportion of *Candida* isolates (49%) was observed in the 21–40 years age group. This pattern is often observed because this age group individuals to be more active socially and have greater exposure to risk factors such as antibiotic use, hormonal fluctuations, and changes in immunity that can facilitate *Candida* colonization.

In the present study, *Candida* species were most frequently isolated from urine samples (33.6%), followed closely by blood samples (30%), reflecting the common occurrence of Candiduria and candidemia in clinical settings **(Fig-6)**. In a study conducted by Sharma F. et al. (2020), *Candida* species were most frequently isolated from urine samples (53.33%), followed by blood samples (21.11%). This finding corresponds with a study by Sugashini D. et al. (2025), in which 48.8% of *Candida* isolates were recovered from urine samples.

in the present study, the highest proportion of isolates were identified as *C. albicans* (57.6%), followed by non-*albicans Candida* (NAC) species including *C. tropicalis* (37.6%), *C. glabrata* (2.8%), and *C. parapsilosis* (2%) **(TABLE-II)**. In various studies, *C. albicans* has been reported as the most predominant *Candida* species. This finding closely corresponds with the study of **Narayankar P. et al (2025)[16]** in which C. albicans accounted for 47% of isolates and C. tropicalis represented 26% of the cases.

In the present study, *Candida* isolates from various clinical samples were identified using conventional methods and CHROMagar Candida differential agar, and the effectiveness of CHROMagar Candida differential agar for species identification was also evaluated, as summarized in **Fig-4**. CHROMagar demonstrated complete sensitivity and specificity (100%) for detecting *C. albicans*, *C. tropicalis*, *C. glabrata*, and *C. parapsilosis*. However, CHROMagar remains a reliable method for the identification of *Candida* species, including in settings with limited resources.

Its ease of use and reliability make CHROMagar a valuable tool for routine clinical diagnosis, particularly where laboratory resources are limited. Early and accurate identification allows clinicians to initiate targeted antifungal treatment promptly, potentially lowering patient morbidity and mortality.

## CONCLUSION

Conventional methods like gram stain, KOH mount, germ tube test, morphology on corn meal agar, sugar fermentation and sugar assimilation tests used for the identification of Candida species up to species level were very time consuming and cumbersome. Female patients accounted for 52.4% and Male patients 47.6% of cases. Women, being in the active reproductive age group, are also susceptible to infection.

The highest prevalence of infection was observed in the 21–40 years age group, indicating that young adults are more commonly affected, followed by the 41–60 years age group, suggesting a gradual decline in susceptibility with increasing age.

The highest number of *Candida* isolates was recovered from urine, with fewer isolates obtained from blood, and the least from high vaginal swabs (HVS), indicating the urinary tract as the most common site of infection in this study.

*C. albicans* was the most frequently isolated species, while non-*albicans Candida* species accounted for the remaining isolates. This highlights that *C. albicans* remains the dominant species in clinical infections, but non-*albicans Candida* species also contribute significantly.

The present study highlights the fact that CHROMagar Candida differential agar is an effective for identifying the four major isolates of Candida species like Candida albicans, Candida tropicalis, Candida krusei and Candida glabrata. CHROMagar provides a simple, rapid, and cost-effective method for the identification of *Candida* species, even in resource-limited settings.

## REFERENCES

1. Dubey D, et al. Exploring rapid molecular methods for diagnosing Candida species infecting humans: A narrative review. Microbes and Infectious Diseases. 2024; 5(1): 336-346.
2. Shwetha DC and Venkatesha D. Speciation of Candida using CHROMagar isolated from Various Clinical



©2026 Int. J. Life Sci. Biotechnol. Pharma. Res

DOI: 10.69605/ijlbpr_15.3.2026.74

Samples. Tropical Journal of Pathology and Microbiology. 2020; 6(4): 303-308.
3. Nirmal K, Nirmal S, Chawla and Baveja CP. Evaluation of HiCrome Candida Differential Agar for Identification of Candida Isolated from Various Clinical Specimens. International Journal of Current Microbiology and Applied Sciences. 2021; 10(8): 341-350.
4. Mishra S, Subramanian A and Kindo AJ. Clinicomycological study of Candida isolates in a tertiary care hospital: A pilot study. Journal of Medical Society. 2021; 35: 58-62.
5. Warghade AP, Mudey G, Meshram S, Kombe S and Shaw D. Characterization and Susceptibility Pattern of Candida Species from Various Clinical Samples in a Rural Tertiary Care Hospital. Journal of Pure and Applied Microbiology. 2023; 17(3): 1880-1886.
6. Shankarnarayan S.A, et al. Molecular Typing and Antifungal Susceptibility of Candida viswanathii, India. Emerging Infectious Diseases. 2018; 24(10): 1956-1958.
7. Dutta G, Verma K, Kaur K and Bareja R. Identification of Candida species from blood samples of neonates by PCR-RFLP method in Western U.P. IP International Journal of Medical Microbiology and Tropical Diseases. 2024; 10(3): 236-239.
8. Parimala TV and Swaruparani. Laboratory Identification of Candida Species from Various Clinical Specimens by Convention Method and Hichrom Agar. IOSR Journal of Dental and Medical Sciences. 2024; 23(7): 56-60.
9. Bayona JVM, Garcia CS, Palop NT and Cardona CG. Evaluation of a novel chromogenic medium for Candida spp. identification and comparison with CHROMagar™ Candida for the detection of Candida auris in surveillance samples. Diagnostic Microbiology and Infectious Disease. 2020; 98: 115-168
10. Sharma S, Kathuria V, Kumar S and Datta S. Evaluation of HiCrome Differential Agar for Species Identification of Candida Isolates from Various Specimens. European Journal of Pharmaceutical and Medical Research. 2020; 7(10): 466-469.
11. Mehta R, and Wyawahare AS. Evaluation of Hicrome Candida Differential Agar for Species Identification of Candida Isolates from Various Clinical Samples. International Journal of Contemporary Medical Research. 2016; 3(4): 1219-1222.
12. Rajput A, Rajput M, Parveen C and Jain V. Prevalence of Candida Infections and Their Resistance Profile Among Patients at Tertiary Care Hospitals, North-India. International Journal of Pharmacy Research & Technology. 2025; 15(2): 1107-1117.
13. Baniodeh H, et al. Phenotypic and genotypic characterization of Candida parapsilosis complex isolates from a Lebanese hospital. BMC infectious disease. 2024; 24: 1142-1150.
14. Solomon DA, Nyerere AK, kanyua A and Ngugi CW. Prevalence, Species Distribution and Antifungal Susceptibility Profile of Candida Species Isolated from Bloodstream of Critical Care Unit Patients in a Tertiary Care Hospital in Kenya. Open Journal of Medical Microbiology. 2021; 11: 32-46.
15. Al Halteet S, Abdel-Hadi A, Hassan M, Awad M. Prevalence and antifungal susceptibility profile relevant candida of clinically species in postmenopausal women with diabetes. BioMed Research International. 2020 Nov 26;2020.
16. Narayankar P, Jayaram A and Manoli PN. Antifungal Susceptibility Pattern of Clinical Isolates of Candida from a Tertiary Care Hospital in Karnataka, India. International Journal of Academic Medicine and Pharmacy. 2025; 7(3): 451- 457.
17. Aruna M and Jahappriya JD. Species Distribution and Antifungal Susceptibility Patterns of Candida Isolates: A Cross-Sectional Study from a Tertiary Care Hospital in South India. Cureus. 2025; 17(2): 1-12.
18. Sachin BK, et al. Prevalence of Candida species and their antifungal susceptibility patterns among patients in tertiary care setting Butwal, Nepal: A cross-sectional study. Asian Journal of Medical Science. 2024; 15(4): 153-159.
19. Sugashini D, Bavadharani S, Anto PV, Sai NV and Santharam P. Phenotypic Characterisation of Candida Species from Various Clinical Sample Obtained in a Tertiary Care Centre in Chengalpattu District. International Journal of Environmental Sciences. 2025; 11(15): 2201-2206.
20. Sugashini D, Bavadharani S, Anto PV, Sai NV and Santharam P. Phenotypic Characterisation of Candida Species from Various Clinical Sample Obtained in a Tertiary Care Centre in Chengalpattu District. International Journal of Environmental Sciences. 2025; 11(15): 2201-2206.



©2026 Int. J. Life Sci. Biotechnol. Pharma. Res