Original Article www.pimr.org.in

DOI: 10.47799/pimr.1301.07

Identification and Speciation of *Aspergillus* in Clinical and Environmental Samples using Culture-Based Methods: A Laboratory-based Study in Hyderabad

Shaik Meherunnisa Begum[1], Mohd Ubaidullah Ansari[2], Mohammed Yasser Arafath K M[3], Juveria Sultana[4]*

[1]Assistant Professor, Department of Microbiology, Osmania General Hospital, Hyderabad, Telangana, India
[2]Assistant Professor, Department of Microbiology, Government Medical College, Bhadradri Kothagudem, Telangana, India
[3]Postgraduate, Department of Microbiology, Osmania Medical College, Hyderabad, Telangana, India
[4]Assistant Professor, Department of Microbiology, Prathima Relief Institute of Medical Sciences, Warangal, Telangana, India

***Corresponding Author:**
Juveria Sultana, Assistant Professor, Department of Microbiology, Prathima Relief Institute of Medical Sciences, Warangal, Telangana, India
E-MAIL: djuveria@gmail.com

COPYRIGHT: ©2025 (Begum) et al. This is an open-access journal, and articles are distributed under the terms of the Creative Commons Attribution License CC-BY 4.0. (https://creativecommons.org/licenses/by/4.0/) which permits unrestricted use, distribution, and reproduction in any medium, provided the original authors and source are credited.

Date of Submission: 26/06/2024 **Date of Review: 02/04/2025** **Date of Acceptance: 10/04/2025**

ABSTRACT

Background: Aspergillosis is increasingly recognized as a serious opportunistic infection, particularly among immunocompromised patients. Accurate species-level identification of *Aspergillus* is critical for selecting appropriate antifungal therapy and implementing effective infection control measures. This study aimed to identify and characterize *Aspergillus* species isolated from both clinical specimens and indoor air samples at a tertiary care hospital in Hyderabad, Telangana, India. **Methods:** Over a one-year period, a total of 709 specimens—including nasal mucosa swabs, tissue biopsies (lung, FESS), blood samples, nail clippings, and passive indoor air settle plates—were processed in the microbiology laboratory. Direct microscopic examination was performed using KOH mount, periodic acid–Schiff stain, and calcofluor white stain. Specimens showing filamentous fungi were cultured on Sabouraud dextrose agar, potato dextrose agar, corn meal agar, Czapek medium, and malt extract agar, and incubated at 25–30 °C for 7–10 days. Species identification was based on detailed assessment of colony morphology and microscopic features (conidiophore structure, vesicle shape, phialide arrangement, and conidial ornamentation), following CLSI guidelines. **Results:** Out of 709 specimens, *Aspergillus* species were isolated from 239 samples (33.7%). Thirteen species were identified, with *A. flavus* being the most common (33.4%), followed by *A. niger* (26.7%), *A. fumigatus* (19.2%), *A. nidulans* (4.6%), *A. glaucus* (3.3%), *A. terreus* (2.9%), *A. versicolor* (2.1%), *A. calidoustus* (1.7%), *A. glabrata* (1.7%), *A. parasiticus* (1.2%), *A. clavatus* (1.3%), *A. ochraceus* (0.8%), and *A. tanneri* (0.8%). *A. flavus* predominated among both clinical and environmental isolates. **Conclusion:** Conventional morphological methods combined with the use of multiple culture media proved effective for species-level identification of *Aspergillus*. Routine surveillance of *Aspergillus* species in both clinical and environmental samples can guide targeted antifungal therapy and support proactive infection control in healthcare environments.

KEYWORDS: *Aspergillus*, Speciation, Differential media, Clinical specimens, Environmental surveillance

INTRODUCTION

The genus *Aspergillus* now comprises of 339 recognized mould species, widely distributed in soil, decaying vegetation, and ambient air. Of these, approximately 40 species are known to cause human disease, ranging from superficial infections such as otomycosis and cutaneous lesions to more severe manifestations like chronic necrotizing and invasive pulmonary aspergillosis, particularly among immunocompromised individuals. [1, 2] The most commonly isolated species in clinical practice include *A. fumigatus, A. niger, A. terreus*, and *A. flavus*. [3]

Aspergillus fumigatus remains the leading cause of invasive aspergillosis worldwide. This is attributed to its small conidial size, which facilitates deep penetration into the respiratory tract, and its thermotolerance. [4, 5] In contrast, *A. flavus* is the predominant species in hot, arid regions

such as the Indian subcontinent, owing to its ability to survive at higher temperatures and its distinct virulence factors, including potent mycotoxin production and strong allergenic potential. [6, 7]

A. terreus also exhibits inherent resistance to amphotericin B [8], which highlights the critical need for accurate species-level identification to guide appropriate antifungal therapy.

Species differentiation in routine microbiology laboratories primarily relies on the assessment of colony morphology including growth rate, surface and reverse pigmentation, and texture as well as microscopic features such as conidial ornamentation and the arrangement of vesicles and phialides. These methods are standardized under the Clinical and Laboratory Standards Institute (CLSI) guidelines (M38-A2). [9]

Balajee et al. demonstrated that careful macroscopic and microscopic examination of isolates grown on Sabouraud dextrose agar, Czapek's solution agar, and malt extract agar enables accurate differentiation of common pathogenic species such as *A. fumigatus, A. niger, A. flavus*, and *A. terreus*. [3]

While molecular techniques such as polymerase chain reaction (PCR) assays targeting species-specific DNA sequences [10] and matrix-assisted laser desorption/ionization–time of flight mass spectrometry (MALDI-TOF MS) for proteomic profiling offer rapid and highly precise identification [11], their implementation requires specialized instrumentation, molecular infrastructure, and skilled personnel, limiting their accessibility in many resource-limited settings. [12] As a result, conventional culture-based methods combined with microscopic evaluation remain vital tools for *Aspergillus* speciation in clinical mycology laboratories.

Environmental surveillance of *Aspergillus* within healthcare facilities is equally important to infection control, given that airborne spores can cause nosocomial outbreaks, particularly during periods of hospital construction or ventilation system failures. Gravity-settle plates and volumetric air sampling methods have demonstrated a strong correlation between elevated airborne fungal counts and the incidence of invasive aspergillosis in high-risk units. [13] Recent reviews emphasize the importance of routine environmental monitoring and the implementation of strict control measures to reduce patient exposure to pathogenic *Aspergillus* species. [14]

The present laboratory-based study was undertaken to identify the species distribution of *Aspergillus* isolates recovered from routine clinical specimens (including nasal mucosa swabs, tissue biopsies, blood samples, and nail clippings) and passive indoor air samples at a tertiary care hospital in Hyderabad, India.

MATERIALS AND METHODS

Study design and setting: This laboratory-based cross-sectional study was conducted in the Microbiology Laboratory of Osmania General Hospital, Hyderabad, Telangana, India, between 1 April 2023 and 31 March 2024. The study aimed to determine the prevalence and species distribution of *Aspergillus* in routinely processed clinical and environmental specimens over a defined one-year period.

Specimen selection: All clinical (nasal mucosa swabs, tissue obtained during functional endoscopic sinus surgery [FESS], lung biopsies, blood, and nail clippings) and environmental (indoor air) specimens submitted for mycological evaluation during the study period were included. No additional sampling was performed outside routine diagnostic submissions.

Air sampling: Passive air sampling was conducted monthly using the settle-plate method in high-risk wards and shared hospital areas. Sterile 90 mm Petri dishes containing Sabouraud dextrose agar (SDA; HiMedia, Mumbai, India) were exposed at a height of 1.5 meters for 30 minutes. Plates were then sealed and processed along with clinical specimens.

Direct microscopy: non-tissue specimens were examined using 10% potassium hydroxide (KOH) wet mounts. Tissue biopsies were assessed using periodic acid–Schiff (PAS) staining and calcofluor white (CFW) fluorescence microscopy to detect filamentous fungal elements.

Culture and incubation: Specimens positive for filamentous fungi on microscopy were inoculated in parallel onto SDA, Potato dextrose agar (PDA), Czapek's solution agar (CZA), and Malt extract agar (MEA), all procured from HiMedia (Mumbai, India). Cultures were incubated at 25 ± 2 °C for up to 10 days, Observations like colony diameter, surface and reverse pigmentation, texture and growth rate—were recorded on Days 3, 5 and 7.

Phenotypic identification: Slide cultures were prepared on CZA and stained with lactophenol cotton blue (LPCB). Microscopic identification was based on conidiophore morphology, vesicle configuration, phialide arrangement, and conidial ornamentation, examined under 400× magnification. Species determination followed CLSI M38-A2 guidelines and standard taxonomic references.

Quality control: *A. fumigatus* ATCC 204305 was included as an internal control to verify culture media performance, incubation parameters, and morphological identification consistency.

Data analysis: Frequencies and species proportions were described in number and percentages and calculated using MS Excel 2021.

RESULTS

A total of 709 specimens were processed over the one-year study period; *Aspergillus* was isolated from 239 samples (33.7%), comprising of 149 (62.3%) clinical specimens and 90 (37.7%) environmental (passive indoor air) samples. Thirteen *Aspergillus* species were identified, with *A. flavus, A. niger*, and *A. fumigatus* collectively accounting for 79.3% of all isolates (Table 1).

Species	Frequency	Percentage (%)
A. flavus	80	33.5
A. niger	64	26.8
A. fumigatus	46	19.2
A. nidulans	11	4.6
A. glaucus	8	3.3
A. terreus	7	2.9
A. versicolor	5	2.1
A. calidoustus	4	1.7
A. glabrata	4	1.7
A. parasiticus	3	1.2
A. clavatus	3	1.3
A. ochraceus	2	0.8
A. tanneri	2	0.8

Table 1: Species distribution of *Aspergillus* isolates (n = 239)

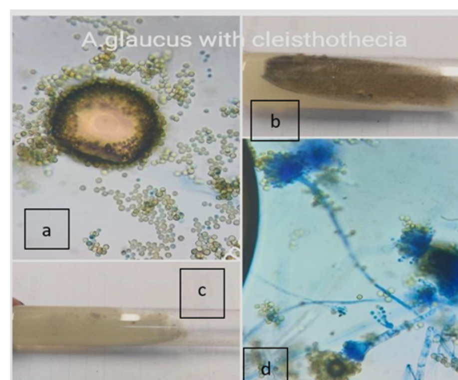

Figure 1: *A. glaucus* showing a) cleistothecia, b) slant obverse, c) slant reverse, and d) microscopy by LPCB stain

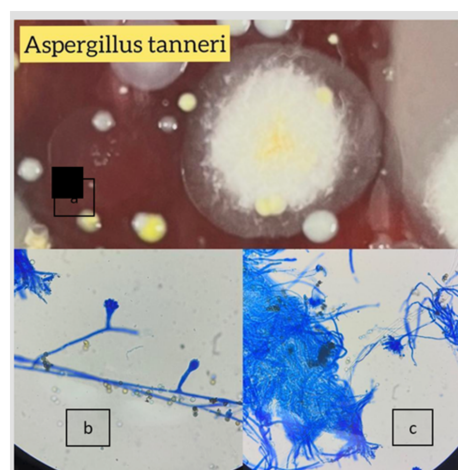

Figure 2: *A. tanneri* showing a) growth on plate and b, c) microscopy by LPCB stain

Macroscopic and microscopic observations

Colonies grown on Sabouraud dextrose agar (SDA), Czapek's solution agar (CZA), and malt extract agar (MEA) exhibited distinct species-specific features such as pigmentation, texture, and growth rate. Microscopic examination revealed characteristic structures like conidiophore branching patterns, vesicle shapes, phialide arrangements, and conidial ornamentation, which were in accordance with CLSI M38-A2 guidelines. Characteristic features of the three most frequently isolated species are summarized in Table 2.

Species distribution by specimen type

The species distribution across the seven specimen categories is shown in Table 3. *A. flavus* was the most frequently isolated species from both clinical specimens (47/149; 31.5%) and environmental air samples (33/90; 36.7%), followed by *A. niger* in both categories.

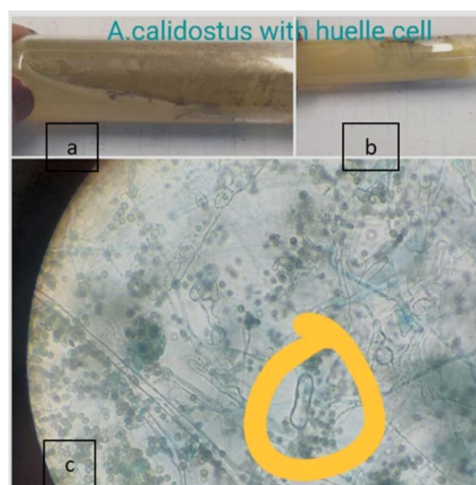

Figure 3: *A. calidoustus* slant showing a) obverse, b) reverse, and c) microscopy showing Hülle cell

Species	Medium	Obverse colour	Reverse colour	Key microscopic features
A. flavus	SDA	Yellow-green	Olive-green	Rough, echinulate conidia; biseriate phialides
	CZA	Yellow-green	Hyaline	
	MEA	Dark green	Hyaline	
A. fumigatus	SDA	Smoky-grey	Green	Smooth, globose conidia; uniseriate phialides
	CZA	Grey	Hyaline	
	MEA	Blue-grey	Hyaline	
A. terreus	SDA	Beige	Cinnamon-buff	Cylindrical, rough-walled conidia; columnar uniseriate phialides
	CZA	Yellow-orange	Light yellow	
	MEA	Light yellow	Yellowish-brown	

Table 2: Macroscopic and key microscopic characteristics of selected *Aspergillus* species

Species	Blood	Nasal mucosa	FESS tissue	Middle meatus	Lung biopsy	Nail clippings	Air samples	Total
A. flavus	1	17	18	7	2	2	33	80
A. niger	0	12	10	11	0	3	28	64
A. fumigatus	1	7	14	8	5	0	11	46
A. nidulans	0	3	1	0	6	0	1	11
A. glaucus	0	1	1	1	2	0	3	8
A. terreus	0	2	1	0	3	0	1	7
A. versicolor	0	0	1	1	2	1	1	5
A. calidoustus	0	0	0	0	1	0	3	4
A. glabrata	0	0	2	0	0	2	0	4
A. parasiticus	0	0	0	0	0	0	3	3
A. clavatus	0	0	1	0	0	0	2	3
A. ochraceus	0	0	0	0	2	0	0	2
A. tanneri	0	0	0	2	0	0	0	2
Total	2	42	47	30	21	8	83	239

Table 3: Distribution of *Aspergillus* species by specimen type (n = 239)

In clinical specimens (n = 149): nasal and FESS samples together accounted for 89/149 (59.7%) of clinical isolates whereas in environmental samples (n = 90): the predominant isolates were *A. flavus* (36.7%) and *A. niger* (31.1%).

The most common species isolated from nasal mucosa was *A. flavus* (n=17) followed by *A. niger* (n=12). FESS samples showed *A. flavus* (n=18) followed by *A. fumigatus* (n=14) and *A. niger* (n=10). Middle meatus showed presence was *A. niger* (n=11) followed by *A. fumigatus* (n=8) and *A. flavus* (n=7). Majority of the species from air samples belonged to *A. flavus* (n=33) followed by *A. niger* (n=28) and *A. fumigatus* (n=11).

DISCUSSION

In this laboratory-based cross-sectional study, *Aspergillus* species were isolated from 33.7% (239/709) of routinely processed clinical and environmental specimens. While direct comparison with large surveillance datasets is limited by differing denominators and inclusion criteria, previous Indian studies report broadly similar species distributions. Bansal et al. [15] documented *A. flavus* (40%), *A. fumigatus*

(35%), and *A. niger* (25%) as predominant isolates in 200 culture-positive clinical samples. Similarly, Chowdhary et al. [16], in a referral chest hospital in Delhi, reported *Aspergillus* isolation in 25.7% of clinical specimens, with *A. flavus* (45.4%), *A. fumigatus* (32.4%), and *A. terreus* (6.6%) being most frequent. Additionally, this finding is consistent with research conducted by Diba K. et al. [17] and Balajee SA et al. [3]

Our finding that *A. flavus* (33.5%) was the most frequently isolated species emphasizes its epidemiological predominance in the tropical Indian context. This may be due to its inherent thermotolerance, environmental resilience, and the production of mycotoxins and allergens that enhance virulence. [6]

Environmental surveillance using passive air sampling recovered *Aspergillus* from 90 samples (37.7% of total isolates), with *A. flavus* (36.7%) and *A. niger* (31.1%) being most common. These results are in consistence with a settle-plate surveillance study from a hospital in Puducherry, where *A. niger* (25.0%), *A. flavus* (17.3%), and *A. fumigatus* (13.5%) were the dominant airborne fungi. [18] Discrepancies in species prevalence may reflect differences in microenvironmental variables such as air circulation, humidity, temperature, and human activity levels. This highlights the importance of standardized environmental surveillance to guide infection-control strategies. [19]

Species-level identification is critical for clinical relevance. Although *A. terreus* accounted for only 2.9% of isolates in our study, its intrinsic resistance to amphotericin B [8] highlights the risk of therapeutic failure when misidentified. Additionally, the emergence of azole-resistant *A. fumigatus*, potentially driven by agricultural fungicide exposure, is of growing concern. A recent Indian study reported a 1.7% prevalence of triazole resistance in clinical isolates [16], stressing the value of routine antifungal susceptibility testing for both clinical and environmental strains.

This study has several limitations. Species identification was based exclusively on morphological criteria without molecular confirmation, which may limit taxonomic precision. Antifungal susceptibility profiling was not performed, excluding assessment of local resistance patterns. Furthermore, passive settle-plate sampling may underestimate airborne spore loads compared to active volumetric methods. Future studies should incorporate advanced diagnostic modalities such as polymerase chain reaction (PCR), matrix-assisted laser desorption/ionization–time of flight mass spectrometry (MALDI-TOF MS), and antifungal susceptibility testing to strengthen epidemiological insights and therapeutic guidance.

In summary, our findings are consistent with the predominance of *A. flavus* in both clinical and environmental settings in India. Despite resource constraints, conventional morphology-based methods remain effective for routine speciation in diagnostic laboratories. However, integrating molecular tools and resistance surveillance into routine practice is essential to enhance patient outcomes and reinforce infection prevention efforts.

CONCLUSION

This laboratory-based cross-sectional study demonstrated that *Aspergillus* species were isolated from one-third of routine clinical and environmental specimens, with *A. flavus, A. niger*, and *A. fumigatus* collectively accounting for nearly 80% of all isolates. The predominance of *A. flavus* in both patient-derived and indoor air samples highlights its epidemiological significance in tropical climates and suggests a potential role in healthcare-associated transmission.

Conventional morphological identification using differential culture media remains a practical and cost-effective method for species-level identification, particularly in resource-constrained settings. However, to improve patient management and strengthen infection control, future initiatives should incorporate active volumetric air sampling, molecular diagnostic techniques (e.g., PCR, MALDI-TOF MS), and routine antifungal susceptibility testing. These measures will enable more precise surveillance, facilitate early detection of antifungal resistance, and guide targeted antifungal therapy.

DISCLOSURE

Funding: None

Conflict of Interest: None Declared

Author Contribution: All the authors involved in study have contributed equally at all stages of work

Acknowledgements: I would like to express my appreciation to all those who have supported and contributed to the completion of this project

REFERENCES

1. Aspergillosis. Atlanta: Centers for Disease Control and Prevention (CDC); 2024. Available from: https://www.cdc.gov/aspergillosis/about/.

2. Samson RA, Visagie CM, Houbraken J, Hong SB, Hubka V, Klaassen CH et al. Phylogeny, identification and nomenclature of the genus Aspergillus. Studies in Mycology. 2014;78(1):141–173. Available from: https://doi.org/10.1016/j.simyco.2014.07.004.

3. Balajee SA, Houbraken J, Verweij PE, Hong SB, Yaghuchi T, Varga J et al. Aspergillus species identification in the clinical setting. Studies in Mycology. 2007;59:39–46. Available from: https://doi.org/10.3114/sim.2007.59.05.

4. Hedayati MT, Mayahi S, Denning DW. A study on Aspergillus species in houses of asthmatic patients from

Sari city, Iran and a brief review of the health effects of exposure to indoor Aspergillus. Environmental Monitoring and Assessment. 2010;168(1-4):481–487. Available from: https://doi.org/10.1007/s10661-009-1128-x.

5. Latgé J, Chamilos G. Aspergillus fumigatus and Aspergillosis in 2019. Clinical Microbiology Reviews. 2019;33(1):1–75. Available from: https://doi.org/10.1128/cmr.00140-18.
6. Rudramurthy SM, Paul RA, Chakrabarti A, Mouton JW, Meis JF. Invasive Aspergillosis by Aspergillus flavus: Epidemiology, Diagnosis, Antifungal Resistance, and Management. Journal of Fungi. 2019;5(3):1–23. Available from: https://doi.org/10.3390/jof5030055.
7. Hedayati MT, Pasqualotto AC, Warn PA, Bowyer P, Denning DW. Aspergillus flavus: human pathogen, allergen and mycotoxin producer. Microbiology. 2007;153(6):1677–1692. Available from: https://doi.org/10.1099/mic.0.2007/007641-0.
8. Lass-Flörl C, Dietl A, Kontoyiannis DP, Brock M. Aspergillus terreus Species Complex. Clinical Microbiology Reviews. 2021;34(4):1–27. Available from: https://doi.org/10.1128/cmr.00311-20.
9. CLSI guideline M54. Principles and Procedures for Detection and Culture of Fungi in Clinical Specimens. 2nd Edition. Clinical and Laboratory Standards Institute. 2021;p. 1–13. Available from: https://cdn.bfldr.com/YLD4EVFU/at/hfk9s9rmh9fn38244c3pc9mx/m54_sample.pdf.
10. Arvanitis M, Ziakas PD, Zacharioudakis IM, Zervou FN, Caliendo AM, Mylonakis E. PCR in diagnosis of invasive aspergillosis: a meta-analysis of diagnostic performance. Journal of Clinical Microbiology. 2014;52(10):3731–3742. Available from: https://doi.org/10.1128/jcm.01365-14.
11. Calderaro A, Chezzi C. MALDI-TOF MS: A Reliable Tool in the Real Life of the Clinical Microbiology Laboratory. Microorganisms . 2024;12(2):1–26. Available from: https://doi.org/10.3390/microorganisms12020322.
12. Wickes BL, Wiederhold NP. Molecular diagnostics in medical mycology. Nature Communications. 2018;9(1):1–13. Available from: https://doi.org/10.1038/s41467-018-07556-5.
13. Iwen PC, Davis JC, Reed EC, Winfield BA, Hinrichs SH. Airborne fungal spore monitoring in a protective environment during hospital construction, and correlation with an outbreak of invasive aspergillosis. Infection Control & Hospital Epidemiology. 1994;15(5):303–306. Available from: https://doi.org/10.1086/646916.
14. Spagnolo AM. Aspergillus Contamination in Healthcare Facilities: An Ever-Present Issue-Prevention and Control Measures. Hygiene. 2025;5(1):1–13. Available from: https://doi.org/10.3390/hygiene5010003.
15. Bansal H, Oberoi L, Pandhi N, Malhotra A, Oberoi T. Species Identification and In Vitro Antifungal Susceptibility Testing of Aspergillus Isolated from Various Clinical Samples. Asian Journal of Pharmaceutical and Clinical Research. 2024;17(10):90–92. Available from: https://doi.org/10.22159/ajpcr.2024v17i10.52052.
16. Chowdhary A, Sharma C, Kathuria S, Hagen F, Meis JF. Prevalence and mechanism of triazole resistance in Aspergillus fumigatus in a referral chest hospital in Delhi, India and an update of the situation in Asia. Frontiers in Microbiology. 2015;6:1–10. Available from: https://doi.org/10.3389/fmicb.2015.00428.
17. Diba K, Kordbacheh P, Mirhendi SH, Rezaie S, Mahmoudi M. Identification of Aspergillus species using morphological characteristics. Pakistan Journal of Medical Sciences. 2007;23(6):867–872. Available from: https://www.pjms.com.pk/issues/octdec207/article/article9.html.
18. Valentina Y, Umadevi S. Phenotypic Detection and Quality Assessment of Indoor Air-Borne Microorganisms Using Passive Air Sampling Technique (Settle Plate) at A Tertiary Care Teaching Hospital in Puducherry. Journal of Pure and Applied Microbiology. 2019;13(1):241–245. Available from: https://dx.doi.org/10.22207/JPAM.13.1.25.
19. Mousavi B, Hedayati MT, Hedayati N, Ilkit M, Syedmousavi S. Aspergillus species in indoor environments and their possible occupational and public health hazards. Current Medical Mycology. 2016;2(1):36–42. Available from: https://doi.org/10.18869/acadpub.cmm.2.1.36.

How to cite this article: Begum SM, Ansari MU, Arafath K M MY, Sultana J. Identification and Speciation of *Aspergillus* in Clinical and Environmental Samples using Culture-Based Methods: A Laboratory-based Study in Hyderabad. Perspectives in Medical Research. ;13(1):29-34
DOI: 10.47799/pimr.1301.07