Asian Journal of Biology

Volume 20, Issue 6, Page 91-97, 2024; Article no.AJOB.117210
ISSN: 2456-7124

Molecular Detection of *Candida aaseri* in Oral Cavity of Immunocompromised Patients

Saad J. Rashak [a,b], Marwan Y. Al-Maqtoofi [a*] and Ahmed A. Burghal [a]

[a] Department of Biology, College of Science, University of Basrah, 61004, Basrah, Iraq.
[b] Directorate of Basrah Education, Basrah, Iraq.

Authors' contributions

This work was carried out in collaboration among all authors. Author SJR carried out the experiments. Authors MYAl-M and SJR wrote the manuscript. Authors MYAl-M and AAB supervised the project and conceived the original idea. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ajob/2024/v20i6418

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/117210

Original Research Article

Received: 17/03/2024
Accepted: 20/05/2024
Published: 28/05/2024

ABSTRACT

The impaired human immune system, resulting from diabetes mellitus, can lead to the transition of *Candida* from a commensal to a pathogenic status, causing oral fungal infections. *Candida aaseri* is a dimorphic yeast with lipolytic activity which has not been previously reported in clinical infections. This study reports an extremely rare oral fungal infection associated with *C. aaseri* in a patient with type II diabetes mellitus. The patient, a 42-year-old female with diabetes mellitus type II from Basrah, Iraq, was and identified with a fungal oral infection. Swab samples were collected from the patient's oral cavity for microbial investigation and cultured on Sabouraud Dextrose Agar (SDA) at 37°C for 48 hours. The results revealed pure yeast growth. Blue colonies were observed on CHROMagar Candida. The genomic DNA of the isolated yeast was extracted for molecular-level

**Corresponding author: E-mail: marwan.almaqtoofi@uobasrah.edu.iq, biotech22zx@gmail.com;*

Cite as: *Rashak, S. J., Al-Maqtoofi, M. Y., & Burghal, A. A. (2024). Molecular Detection of Candida aaseri in Oral Cavity of Immunocompromised Patients . Asian Journal of Biology, 20(6), 91–97. https://doi.org/10.9734/ajob/2024/v20i6418*

diagnosis, utilizing the mRNA gene's internal transcribed spacer (ITS) region. *C. aaseri* was the sole yeast species identified in this clinical case. Anti-fungal sensitivity tests indicated that *C. aaseri* was sensitive to Nystatin, Posaconazole, and Fluconazole while exhibiting significant resistance to Colistin Sulfate. This study represents the first global report of isolating *C. aaseri* from an oral infection in an immunocompromised patient.

Keywords: Antifungal; Candida; fungi; oral infection; immunocompromised; ITS; diabetes; Basrah.

ABBREVIATIONS

SDA : Sabouraud Dextrose Agar
ITS : Internal Transcribed Spacer
DCs : Dendritic Cells
NK : Natural Killer
RBS : Random Blood Sugar
NCBI : National Center for Biotechnology Information
CS : Colistin Sulphat

1. INTRODUCTION

The human oral cavity is a unique site that becomes colonized with many microbes, such as fungi, bacteria, and viruses. Among these microbes, *Candida* species are the most frequent colonizers in the human oral cavity that adapted to exist harmlessly within mucus membranes. Overgrowth of *Candida* in the oral cavity can result in various clinical symptoms such as discomfort, pain, changes in taste perception, and difficulty in swallowing (dysphagia) [1]. *C*. *albicans* is considered the most predominant opportunistic yeast for causing candidiasis compared with other species such as *C. tropicalis*, *C. glabrata*, *C. pseudotropicalis*, *C. guillierimondii*, *C. krusei*, *C. lusitaniae*, *C. parapsilosis C. stellatoidea* due to its ability to biofilm and hyphae form and produce hydrolytic enzymes and candialysin [2]. *Candida* species members have the ability to cause infection due to bosses a wide range of virulence factors, such as the expression of adhesions and invasions on the cell surface, the morphological transition between yeast and hyphal forms, thigmotropism, biofilms formation, and the secretion of hydrolytic enzymes [3].

The commensalism relation between the host and *Candida* species bases immune status for both innate and adaptive immune systems [4]. The status of the immune system is key to developing fungal infections. Among immunocompromised individuals, the infection can spread through the bloodstream or upper digestive system, causing a severe infection with higher risks of illness that is associated with an increase in the mortality rate [5]. Diabetic patients are more susceptible to the development of various oral infections, including bacterial and fungal infections, due to the decreasing salivary flow rate and the absence of antimicrobial effects. In addition, poor metabolic control, an impaired defence mechanism, and a defect in the immune system may play an important role in developing infection [6]. For this reason, the rate of *Candida* colonization increases with immunocompromised individuals who suffer from diabetes mellitus and become a serious pathogenic fungus, leading to disseminated candidiasis [7]. Therefore, early diagnosis of pathogenic fungal infections results in prevention and management treatment that is key to removing or alleviating the predisposing conditions [8].

The oral mucosa is composed of both oral epithelial cells, keratinocytes and immune cells, including cells such as macrophages, dendritic cells (DCs), natural killer (NK) cells and polymorphonuclear neutrophils, which contribute to the plethora of immunomodulatory cytokines found in the oral cavity and the production of biological mediators such as antimicrobial peptides [9]. There are four classes of antifungals that differ in their mechanisms of action: polyene amphotericin B which has a broad spectrum and a potent fungicidal effect [10]. Fluorocytosine (flucytosine) inhibits fungal protein synthesis and is also a potent inhibitor of fungal DNA synthesis through the inhibition of thymidylate synthetase [11]. Systemic azoles and currently widely used triasoles have several antifungal formulations, including fluconazole, itraconazole, voriconazole, posaconazole, ravuconazole, and isavuconazole. Azoles inhibit lanosterol 14α demethylase, a key enzyme of ergosterol biosynthesis and has a fungistatic activity against *Candida* spp. [12]. Echinocandins (micafungin, anidulafungin, caspofungin) present the newest class of antifungals and feature a fungicidal effect in Candida species [13]. Some studies focus on using natural products for treating *Candida* infections using honey [14] or

chemically synthesized antimicrobials [15] with no side effects. *C. aaseri* is commonly known as a saprophytic yeast on many plants, such as oil palms [16]. The only report of isolation *of C. aaseri* and closely related species *C. pseudoaaseri* from the sputum of Norwegian patients [17]. There is currently no global research addressing the identification of dimorphic *C. aaseri* as a potential etiological agent of oral infections in an immunocompromised patient with diabetes mellitus type 2 in Basrah, Iraq.

2. METHODS

2.1 Samples Collection

Swab samples from patients with clinically confirmed cases of fungal oral cavity infections were collected from a 42-year-old woman with a five-year history of Diabetes mellitus II presented at A-Sadr Teaching Hospital in Basrah, Iraq, in January 2021. After obtaining ethical approval and reviewing the patient's medical history, it was noted that she was taking Glucophage 500 mg twice daily for her Diabetes. She had not taken any antibacterial or antifungal medications for more than a week, and she did not have any immunosuppressive conditions other than Diabetes. Furthermore, she did not have any heart, liver, or renal diseases, and she did not consume alcohol or smoke. Upon examination, the patient's tongue appeared white with red spots at the edges. A random blood sugar test (RBS) revealed a 300 mg/dL blood sugar level. Oral swab samples were collected and cultured on Sabouraud Dextrose Agar (SDA) at 37°C for 48 hours to investigate the microbial composition.

2.2 Molecular Identification of Isolated Endophytic Fungi

Recovered yeast on Cornmeal tween 80 agar for one week at 25°C appeared as colonies with the ability to produce pseudo-hyphae, but no chlamydospores were formed. No germ tube was formed when grown in human serum for three hrs. at 37 °C. Molecular identification using the ITS regions of the rRNA-encoding region showed that *C. aaseri* was the only microbial colony recovered from this patient using the primers ITS5 5'-TCCGTAGGTGAACCTGCGG-3' and ITS4 5'-TCCTCCGCTTATTGATATGC-3'. PCR amplification condition of the ITS region was conducted according to procedures described by Al-Maqtoofi and Thornton [18]. DNA sequence data were aligned for comparison with those stored in the National Center for Biotechnology Information (NCBI) databases using NCBI BLAST® (https://blast.ncbi.nlm.nih.gov). Phylogenetic analyses were conducted in MEGA 11 [19].

3. RESULTS

A yeast colony was isolated and cultured on CHROM agar™ Candida at 37°C for 48 hours. This resulted in the recovery of distinctive blue colonies, and the letter C3 was used to indicate this isolate (Fig.1). After genomic analysis, the data sequence of isolate (C3) was deposited to GenBank for Accession Number verification (OR398895).

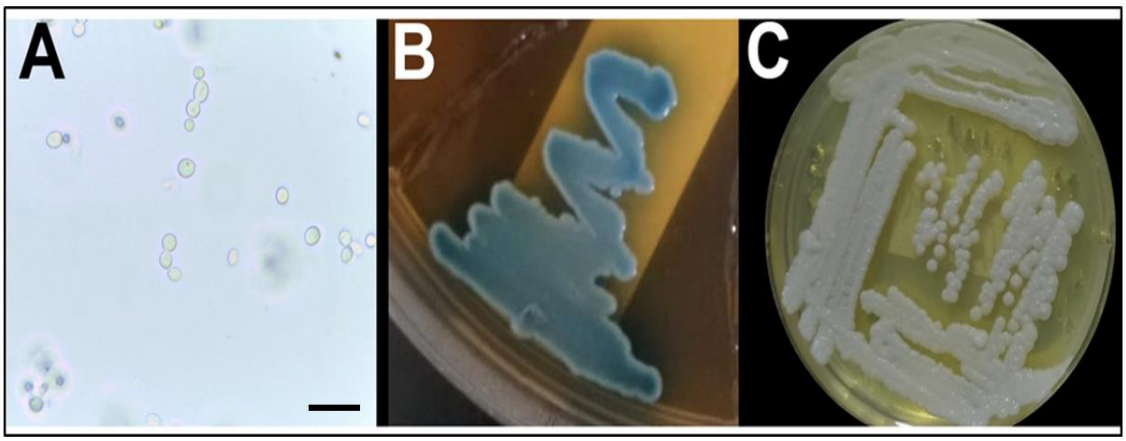

Fig. 1. *C. aaseri* under light microscope. A. *C. aaseri* (100x). B: *C. aaseri* on CHROMagar™ Candida; C: *C. aaseri* on Sabouraud Dextrose Agar. Scale bar= 10 µm

A combination of bioinformatics tools was employed for initial editing and analysis to construct the phylogenetic tree. The analysis result was compatible with *C. aaseri* with per cent identity (100 – 99.43%) (Fig. 2). Antifungal sensitivity test showed that *C. aaseri* was significantly sensitive to fluconazole and posaconazole followed by nystatin (43, 41, 23 mm, inhibition zone) respectively, while exhibiting a notable resistance to Colistin sulphate (Fig. 3).

4. DISCUSSION

Candidiasis infections are a significant concern among immunocompromised individuals who have an impaired immune system, making them more susceptible to opportunistic infections, including those caused by *Candida* species leading to a high morbidity and mortality rate exceeding 70% [20]. The incidence of disseminated candidiasis has an increasing trend

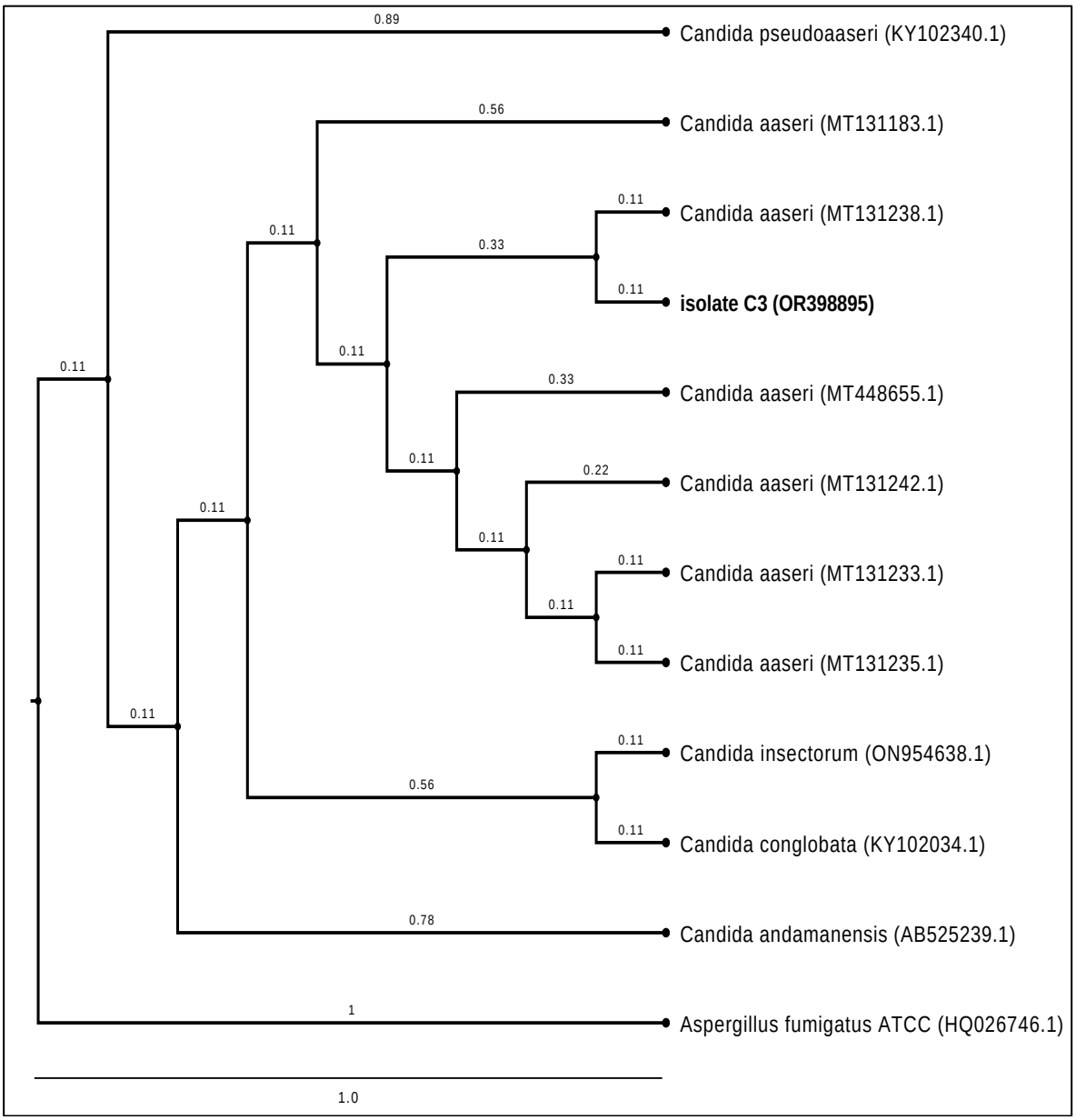

Fig. 2. Phylogenetic tree drawn from neighbour-joining analysis of ITS domain sequences depicting the relationships of *Candida aaseri* (C3) (OR398895) with closely related *Candida* species. Bootstrap percentages over 50% from 1000 bootstrap replicates are shown. Reference sequences were retrieved from GenBank with the accession numbers indicated. The phylogenetic tree was rooted with *Aspergillus fumigatus* as an outgroup

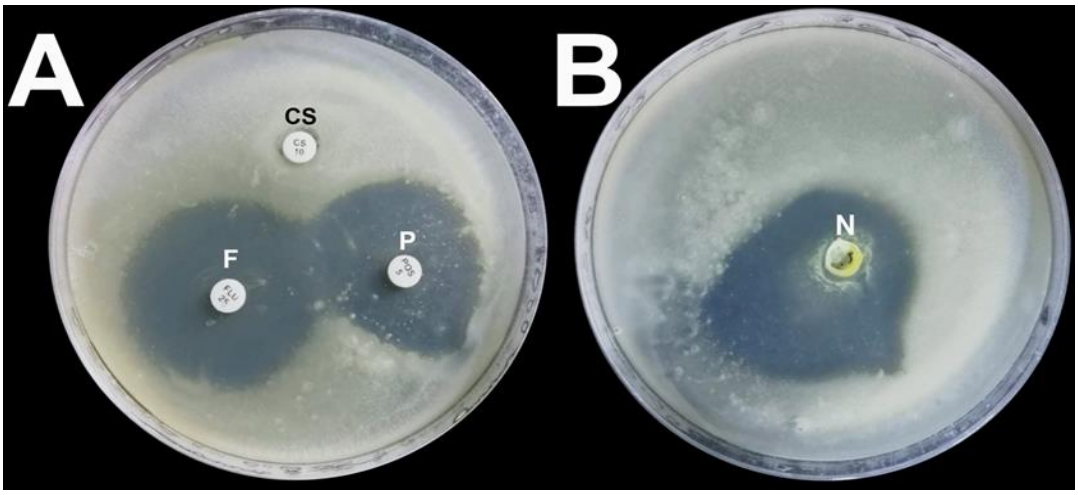

Fig. 3. Antifungal susceptibility test on Mueller-Henton agar for *C. aaseri* where A: Antifungal susceptibility test Fluconazole (F) 25 µg, Posaconazole (P) 5µg and Colistin Sulphate (CS) 10µg; and B: Antifungal susceptibility test of Nystatin (N) 0.1 mg

along with the ever-expanding populations of immunocompromised such as those with diabetic patients [21]. Among the pathogenic species, *C. albicans* is the prevailing and frequently encountered *Candida* species causing candidiasis in human immunodeficiency individuals followed by *C. glabrata, C. krusei, C. parapsilosis* and *C. tropicalis* [22]. In Basrah, a study reported a very rare eye infection by yeast, *Hanseniaspora uvarum* [23]. In a unique clinical case, an extremely rare *Candida* infection was reported in the oral cavity of an immunocompromised patient with diabetes mellitus in Basrah, Iraq. This particular infection is associated with *C. aaseri*. This species of *Candida* has not been reported as a human fungal pathogen. To our knowledge, this study is the only one that showed isolation, identification and successful in-vitro treatment of *C. aaseri* from oral infection of immunocompromised patients with diabetes mellitus in the world.

5. CONCLUSIONS

Candida aaseri has not been reported as a human fungal pathogen. To our knowledge, this study is the only study that showed isolation, identification and successful in-vitro treatment of *C. aaseri* from oral infection of immunocompromised patient with diabetes mellitus in the world.

CONSENT

Written informed consent was obtained prior to the interview.

ETHICS APPROVAL

The study was approved by the University of Basrah, College of Science, Department of Biology Ethic Committee

ACKNOWLEDGEMENTS

The authors are grateful to the University of Basrah, College of Science and the head of Biology department, University of Basrah, Iraq and A-Sadr Teaching Hospital in Basrah, Iraq for providing facilities.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lu SY. Oral Candidiasis: Pathophysiology and Best Practice for Diagnosis, Classification, and Successful Management. Journal of Fungi. 2021;7(7): 555.
Available:https://doi.org/10.3390/jof7070555
2. Patel M. Oral Cavity and *Candida albicans*: Colonisation to the Development of Infection. Pathogens (Basel, Switzerland). 2022;11(3):335.
Available:https://doi.org/10.3390/pathogens11030335
3. Silva DM, Souza TC, Alencar CF, Souza IS, Bandeira MF, Fernandes OC. Virulence

factors of *Candida* species from the oral mucosa and prostheses of elderly people from a riverside community in the Amazon state, Brazil. Revista de Odontologia Da UNESP. 2019;48, e20190094.
Available:https://doi.org/10.1590/1807-2577.09419

4. Mei-Sheng RM. Invasive Fungal Infections Among Immunocompromised Patients in Critical Care Settings: Infection Prevention Risk Mitigation. Critical Care Nursing Clinics of North America. 2021;33(4):395–405.
Available:https://doi.org/10.1016/j.cnc.2021.07.002
5. Jerônimo LS, Esteves Lima RP, Suzuki TY, Discacciati JA, Bhering CL. Oral Candidiasis and COVID-19 in Users of Removable Dentures: Is Special Oral Care Needed? Gerontology. 2022;68(1):80–85.
Available:https://doi.org/10.1159/000515214
6. Rohani B. Oral manifestations in patients with diabetes mellitus. World Journal of Diabetes. 2019;10(9):485–489.
Available:https://doi.org/10.4239/wjd.v10.i9.485
7. Contaldo M, Di Stasio D, Romano A, Fiori F, Della Vella F, Rupe C, Lajolo C, Petruzzi M, Serpico R, Lucchese A. Oral Candidiasis and Novel Therapeutic Strategies: Antifungals, Phytotherapy, Probiotics, and Photodynamic Therapy. Current Drug Delivery. 2023;20(5):441–456.
Available:https://doi.org/10.2174/1567201819666220418104042
8. Al-Rifaie AA, Al-Maqtoofi MY. Immunodetection and risk assessment for *Aspergillus* contamination in nuts using a highly specific monoclonal antibody. Biomedical Research. 2018;29(21).
Available:https://doi.org/10.4066/biomedicalresearch.29-18-1098
9. Casaroto AR, Silva RA, Salmeron S, Rezende ML, Dionísio TJ, dos Santos CF, Pinke KH, Klingbeil MF, Salomão PA, Lopes MM, Lara VS. *Candida albicans*-Cell Interactions Activate Innate Immune Defense in Human Palate Epithelial Primary Cells via Nitric Oxide (NO) and β-Defensin 2 (hBD-2). Cells. 2019;8(7):707.
Available:https://doi.org/10.3390/cells8070707
10. Carolus H, Pierson S, Lagrou K, Van Dijck P. Amphotericin B and Other Polyenes-Discovery, Clinical Use, Mode of Action and Drug Resistance. Journal of Fungi (Basel, Switzerland). 2020;6(4):321.
Available:https://doi.org/10.3390/jof6040321
11. Barantsevich N and Barantsevich E. Diagnosis and Treatment of Invasive Candidiasis. Antibiotics (Basel, Switzerland). 2022;11(6):718.
Available:https://doi.org/10.3390/antibiotics11060718
12. Hassanmoghadam F, Shokohi T, Hedayati MT, Aslani N, Haghani I, Nabili M, Lotfali E, Davari A, Moazeni M. High prevalence of itraconazole resistance among *Candida parapsilosis* isolated from Iran. Current Medical Mycology. 2019;5(3):43–46.
Available:https://doi.org/10.18502/cmm.5.3.1746
13. Ham YY, Lewis JS, Thompson GR. Rezafungin: A novel antifungal for the treatment of invasive candidiasis. Future Microbiology. 2021;16(1):27–36.
Available:https://doi.org/10.2217/fmb-2020-0217
14. Albosolemy T, Hamzah AF, Ahmed AB, Tamimi WH. Biological Activities of Natural Honey Produced in Basrah Governorate. Journal of Pharmaceutical Negative Results. 2022;886–892.
Available:https://doi.org/10.47750/pnr.2022.13.S03.136
15. Yossra AM, Al-Rifaie AA, Wijdan HA, Hayat HA. Synthetic approach to novel pyrimido-triazines with antimicrobial activity. 2019;21(3)
16. Lee SH, Jeong H, Ko HJ, Bae JH, Ibrahim ZH, Sung BH, Sohn JH. Draft Genome Sequence of a Lipolytic Yeast, *Candida aaseri* SH-14. Genome Announcements. 2017;5(20):e00373-17.
Available:https://doi.org/10.1128/genomeA.00373-17
17. Pfüller R, Gräser Y, Erhard M, Groenewald M. A novel flucytosine-resistant yeast species, *Candida pseudoaaseri*, causes disease in a cancer patient. Journal of Clinical Microbiology. 2011;49(12):4195–4202.
Available:https://doi.org/10.1128/JCM.05090-11
18. Maqtoofi MY, Thornton CR. Detection of human pathogenic Fusarium species in hospital and communal sink biofilms by using a highly specific monoclonal

antibody. Environmental Microbiology. 2016;18(11):3620–3634.
Available:https://doi.org/10.1111/1462-2920.13233

19. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Molecular Biology and Evolution. 2018;35(6):1547–1549.
Available:https://doi.org/10.1093/molbev/msy096
Chavda VP, Mishra T, Kamaraj S, Punetha S, Sengupta O, Joshi Y, Vuppu S, Vaghela D, Vora L. Post-COVID-19 Fungal Infection in the Aged Population. 2023;11(3). Article 3.
Available:https://doi.org/10.3390/vaccines11030555
20. Abdoli A, Falahi S, Kenarkoohi A. COVID-19-associated opportunistic infections: A snapshot on the current reports. Clinical and Experimental Medicine. 2022;22(3):327–346.
Available:https://doi.org/10.1007/s10238-021-00751-7
21. Miruka HN, Eric OO, Musyoki S, Awuor SO. Incidence, antifungal resistance properties, and virulence traits of *Candida* species isolated from HIV/AIDS Patients from the hospital system in Kenya. Access Microbiology; 2023.
Available:https://doi.org/10.1099/acmi.0.000620.v1
22. Asaad Aziz Israa, Al-Maqtoofi MY, Ahmed AB. Novel record of fungal eye infection caused by *Hanseniaspora uvarum* and associated with metal splinters. Journal of the Association of Medical Microbiology and Infectious Disease Canada. 2022;*7* (Suppl):1–131.
Available:https://doi.org/10.3138/jammi.7.s1.abst

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/117210