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An uncommon oral infection in a diabetic patient

Arpan K Shah, Meena M Kulkarni[1], Smita U Khedkar[2], Mahesh H Gabhane[3]
Department of Oral Pathology and Microbiology, Manubhai Patel Dental College and Hospital, Vadodara, Gujarat, [1]Department of Oral Pathology and Microbiology, Rural Dental College, Loni, [2]Department of Microbiology, MGV's Karmaveer Bhausaheb Hiray Dental College and Hospital, Nashik, [3]Department of Oral Pathology and Microbiology, Sau. Mathurabai Bhausaheb Thorat Dental College and Hospital, Sangamner, Maharashtra, India

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CASE REPORT

A 53-year-old female reported with burning sensation in lower right posterior region of oral cavity since 2 years. Her mandibular molars from right side were extracted due to mobility, resulting from generalized chronic periodontitis, before 3 years. She had been given a removable partial denture to replace the missing teeth. However, she was unable to wear it due to burning sensation. Intraoral examination revealed a diffuse erythematous lesion involving the mucosa of lower posterior edentulous alveolus on right side.Patient was a known case of type II diabetes mellitus and was under medications for the same.With provisional diagnosis of erythematous candidiasis, the surface material was collected by rubbing sterile swab on the lesion.

Microbial morphology and cultural characteristics

A microbial smear was prepared of the collected material and stained with Gram stain for microscopic examination. On microscopic examination, numerous Gram positive nonbranching septate hyphae, few epithelial cells and debris were observed [Figure 1].

Suspension of the collected material was prepared by agitating the swab in few drops of saline. This suspension was inoculated on the culture medium, "Sabouraud's dextrose agar with chloramphenicol", for confirmation. Inoculation of the specimen was done in laminar hood to avoid the environmental contamination. After incubation at room temperature for 24 h, microscopic examination of the colonies revealed numerous entangled hyphae and immature contiguous spores [Figure 2]. At the end of 48 h, white circular colonies with fine granular or powder-like surface were observed [Figure 3]. The colonies were emulsified when touched with sterile microbiological loop. Gram stained smear of the material obtained from colonies showed numerous cylindrical or barrel-shaped arthrospores [Figure 4]. A wet unstained smear was prepared and examined under microscope, which also showed rectangular arthroconidia [Figure 5].

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Microbiological differential diagnosis and identification by biochemical test

Medically important fungi, which produce septate hyphae and arthroconidia, are Geotrichum spp, Trichosporon spp and *Coccidioides immitis*.[1] Among these, *Coccidioides immitis* causes serious invasive mycosis, while other two cause superficial infections.[1-3] Thus, clinical features, morphology and culture can effectively distinguish coccidioidomycosis. Trichosporon spp more commonly causes infection of skin. Geotrichum species are occasionally involved in superficial mycosis involving oral mucosa.[4-6] Geotrichum and Trichosporn closely resemble in morphology and cultural characteristics. Urease test can demarcate these two pathogenic fungi, where Trichosporon is able to produce urease and Geotrichum does not produce it.[1]

Clinical features, morphological examination and cultural characteristics in present case suggested the possibility of Geotrichum or Trichosporon species. To differentiate these two, urease test was carried out, which was negative [Figure 6]. Based on microscopic, cultural characteristics and biochemical reaction the fungus was identified to be from *Geotrichum* species.

Final diagnosis based on molecular identification by deoxyribonucleic acid sequencing

Fungal genomic DNA was isolated from the culture. Using consensus primers 18s ribosomal ribonucleic acid (rRNA), 5.8 rRNA, 28s rRNA and ITS 1 and ITS 2 gene fragment was amplified using high-fidelity polymerase chain reaction (PCR) polymerase. The PCR product was cloned and sequenced using the forward, reversed and internal primers. Based on nucleotide homology and phylogenetic analysis, the microbe was detected to be *Geotrichum candidum*.

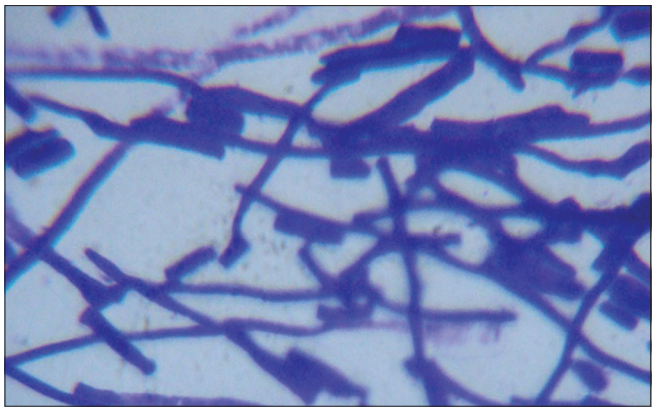

Figure 1: Photomicrograph showing gram positive, nonbranching, septate hyphae (Gram stain, ×1000)

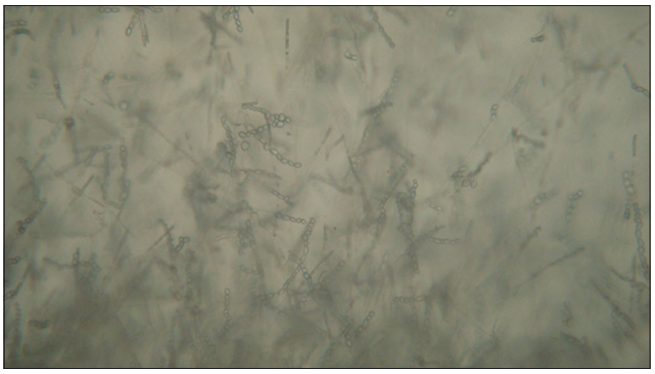

Figure 2: Photomicrograph showing entangled hyphae and contiguous immature arthroconidia (Sabouraud's dextrose agar with chloramphenicol, ×450)

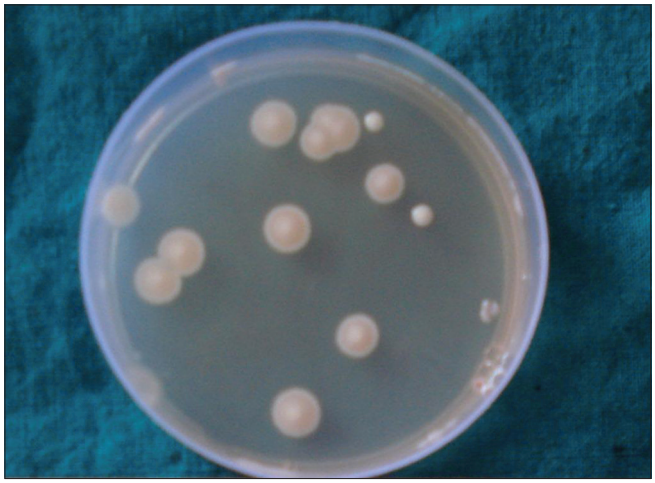

Figure 3: Culture on Sabouraud's medium showing circular, white, flat colonies

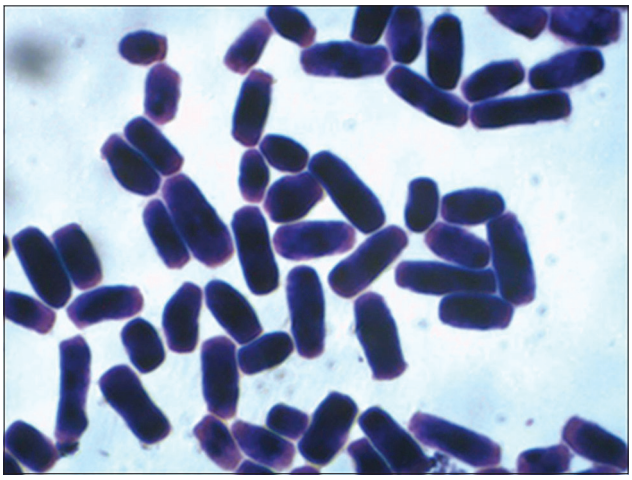

Figure 4: Photomicrograph showing gram positive, barrel-shaped arthrospores (Gram stain, ×1000)

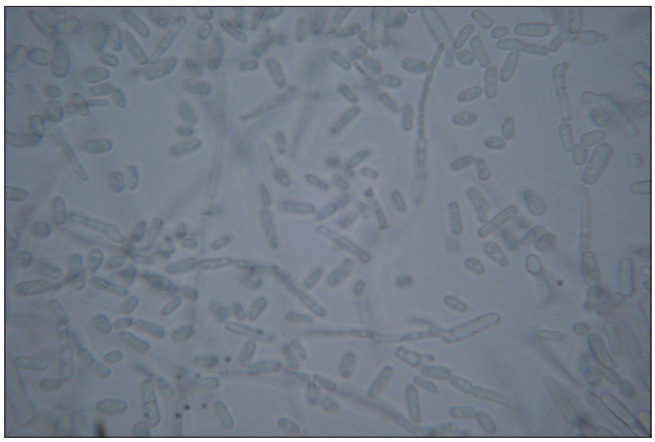

Figure 5: Photomicrograph showing rectangular arthrospores with rounded ends (wet unstained smear, ×450)

Figure 6: Urease test: Urease test of control specimen (Trichosporon spp) showing pink color (positive test), while urease test of present case showing negative result

Antifungal susceptibility testing and treatment

The organism was found to be sensitive to clotrimazole amphotericin-B and nystatin. The infection was resolved after topical clotrimazole treatment for 7 days.

DISCUSSION

Geotrichum candidum is a yeast-like fungus which, similar to *Candida albicans*, forms a part of normal microbial flora in

upper respiratory and gastrointestinal tracts.[4] The infection caused by Geotrichum species of fungi is known as geotrichosis. It is an uncommon infection which, in most cases, is associated with compromised immune system of host, such as diabetes mellitus, acquired immunodeficiency syndrome, patients with organ transplantation and hematolymphoid malignancies.[4-8] Clinically, the infection caused by Geotrichum is similar to candidiasis. It has been described in three clinical varieties viz pseudomembranous, hyperplastic and ulcerative. It is a superficial fungal infection with chronic inflammatory response in underlying connective tissue.[2,4]

In the specimens collected from sites of infection, *Geotrichum candidum* appears in vegetative mycelial form, that is, hyphae or monomorphic molds. Hyphae of *Geotrichum candidum* are hyaline, septate and nonpigmented. The organism reproduces by segmentation of hyphae through the points of septation, with production of multiple oblong or rectangular spores with rounded ends, which are known as arthrospores, arthroconidia, annelloconidia or clavata cells.[1,4] These barrel-shaped arthrospores measure 4–8 μm in length and 4–10 μm in diameter. The arthroconidia easily disperse into the environment.[1]

The fungus usually produces creamy white, smooth, shiny and convex colonies similar to candida. Some isolates produce colonies similar to molds with fine granular or powdery surface.[1] These dry appearing colonies emulsify when touched with microbiological loop, which is a clue to identification of this fungal species.

CONCLUSION

Geotrichosis may be more common than generally assumed. It may be frequently misdiagnosed as candidiasis due to similarities of clinical features and response towards anticandidal treatment. However, it should be considered in the differential diagnosis of oral mucosal infections in immunocompromised patients. Careful microscopic examination and thorough evaluation of cultural characteristics are sufficient to identify Geotrichum species and to differentiate from other fungal species. Antifungal susceptibility testing may become necessary for appropriate treatment of such lesions.

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