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*Case Report*

# Itraconazole-resistant *Candida auris* with phospholipase, proteinase and hemolysin activity from a case of vulvovaginitis

Dharmendra Kumar, Tuhina Banerjee, Chandra Bhan Pratap, Ragini Tilak

*Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India*

**Abstract**

Since the emergence of pathogenic non-*albican*s Candida species, a number of new isolates have been added to the list. One such unusual species is *Candida auris* (*C. auris*), recently isolated and studied in few reports. In this study, a case of vulvovaginitis caused by *Candida auris* incidentally identified by molecular methods using internal transcribed spacer polymerase chain reaction (ITS PCR) is described. Antifungal susceptibility testing revealed the isolate to be resistant to itraconazole (MIC ≥ 2 µg/ml) and expressed important virulence factors including phospholipase, proteinase and hemolysin activity. The patient was successfully treated with oral fluconazole and did not have any invasive fungemia.

Very few cases of this emerging pathogen have been reported. However, its isolation from clinical specimens reveals the significance of non-*albicans* candida species over *C. albicans* and the diversity of *Candida spp* causing infections.

**Key words:** *C. auris*; non-*albicans*; resistance; phospholipase.

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## Introduction

Since the first report on *Candida auris* from Japan in 2009[1], infrequent reports of this species from clinical specimens have been documented. Emergence of non-*albicans Candida spp* as pathogens arebeing increasingly reported from different parts of the world [2], thus emphasizing the importance of identification of *Candida* to a species levelin all laboratories. At the same time, these emerging species also exhibit varying drug susceptibility profiles. Therefore, understanding drug susceptibility of these different species can help develop protocols for appropriate empirical treatment of these infections. In this study we report a case of vulvovaginal candidiasis caused by *C. auris*, identified by molecular methods, along with the characterization of the drug resistance profile and virulence traitsof the isolate.

## Case Report

A 28-year-old woman with complaint of vaginal discharge, burning itchy sensation along with dyspareunia and low back pain presented as outpatient at the Department of Gynaecology, Sir Sunderlal Hospital, a tertiary care university hospital in Varanasi, north India. A clinical diagnosis of vulvovaginitis was made. A bacterial and fungal culture analysis was requested. Two high vaginal swabswerecollected aseptically with the help of a speculum and a post vaginal wall retractor and sent immediately to the Microbiology department for further analysis.

A Gram stained smear was prepared and examined, which showed the presence of ovoid budding yeast cells. The sample was inoculated on Sabouraud's dextrose agar (SDA) and incubated at 37°C for 5 days for fungal culture and blood agar and MacConkey agar for bacterial culture. Following incubation, dull white to cream coloured, smooth colonies were seen on SDA, showing globose budding yeast cells on microscopic examination (Figure 1A). Bacterial culture was negative. For further speciation, germ tube and chlamydospore formation on Cornmeal agar, sugar assimilation and fermentation tests and growth on CHROM agar were performed [3]. Antifungal susceptibility testing was performedby disc diffusion testing using fluconazole (25 µg), itraconazole (10 µg), voriconazole (1 µg) and amphotericin B (100 units/disc) (Hi Media Labs, Mumbai, India); determination of minimum inhibitory concentration (MIC) against the sameantifungals was performed based on standard protocol [4].

The isolate showed no germ tube and chlamydospore formation, produced no characteristic colour on CHROMagar (Figure 1B). Species identification by sugar assimilation and fermentation tests were inconclusive. The isolate grew at 42°C but failed to grow at 45°C. Due to unavailability of automated methods and a known confirmatory method, molecular identification methods by amplifying a conserved portion of 18SrDNA region, adjacent ITS1and a portion of the 28SrDNA were performed,as described elsewhere [5]. On visualization, a band of approximately 479 bp (which was slightly lower than that for *C. glabrata*) was seen (Figure 2). Polymerase chain reaction products were extracted and purified (extraction kit, Qiagen Inc., Valencia, USA) and subjected to DNA sequencing (Genei, Bangalore, India) followed by sequence analysis using BLAST suite programs and compared with the gene bank databases of the national center for biotechnology information (www.ncbi.nl.nih.gov/entrez). The isolate showed greater than 99% similarity to *C. auris* and was identified as such.

Due to infrequent isolation of this species from clinical specimens and in order to reveal its pathogenic nature, the isolate was further studied for virulence determinants as detailed elsewhere [6]. Briefly, determination of phospholipase, proteinase and hemolysin activity was carried out. For phospholipase detection, 5µl of standard yeast suspension ($10^8$ yeast cells/ml) was dropped over egg yolk agar media and air-dried, followed by incubation at 37°C for 48 hours. Phospholipase activity was expressed as $P_z$ value which is the ratio of the diameter of the colony to the diameter of the colony plus the precipitation zone measured in mm. For proteinase activity, $Pr_z$ value was measured as the ratio of the diameter of the colony to that of the clear zone of proteolysis following inoculation on 1% bovine serum albumin (BSA) plate and staining and destaining after 5 days incubation. Hemolysin activity was detected on SDA with 3% glucose and 7% sheep blood and expressed as hemolytic index ($H_z$).*Candida albicans* ATCC 90028 was used as positive control.

The isolate was resistant to itraconazole (MIC≥ 2 µg/ml) and sensitive to fluconazole (MIC ≤16µg/ml), voriconazole (MIC≤0.5µg/ml)and amphotericin B (MIC≤0.5µg/ml). The isolate showed phospholipase activity ($P_z$ value 0.72), proteinase activity ($Pr_z$value 0.66) and hemolysin activity ($H_z$ value 0.74). However, the patient responded to oral fluconazole treatment, without any event of breakthrough fungemia.

**Figure 1. A**. Globose yeast cells in Gram's staining; **B**. *C.auris C.auris* growth on CHROMagar

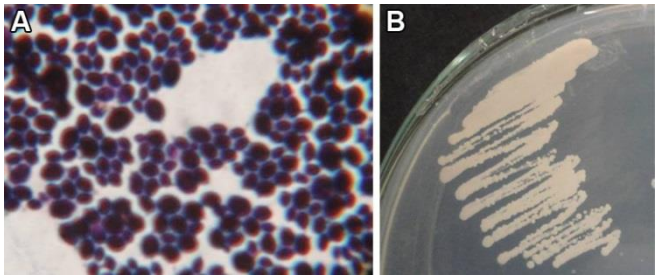
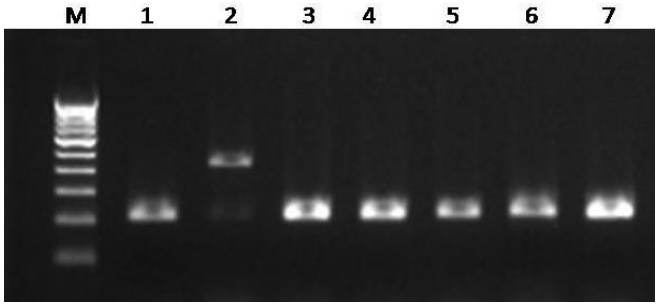



**Figure 2.** PCR amplification product of *Candida spp*




## Discussion

Candidal vulvovaginitis is a worldwide problem, being the second most common cause of vaginitis, affecting millions of women especially in the reproductive age group [7]. Previous studies on vulvovaginitis cases due to *Candida spp* have already documented the increasing prevalence of non-*albicans Candida spp* over *C. albicans*. Indiscriminate use of antifungal agents along with incorrect dosage schedules, prolonged hospitalization, poor immune status are some of the implicated factors behind the emergence of unusual species [8].

There have been a handful of reports on *C. auris* as a pathogen causing fungemia [9,10] but in this case *C. auris* was found as the causative agent for vulvovaginitis. The increasing isolation of *C. auris* from various clinical specimens clearly indicates its ability to colonise, invade and cause disease with varying severity. Though a limited number of studies have addressed this isolate, the majority of them have reported this species to be intrinsically resistant to azoles and amphotericin B [11]. *C. auris* is intrinsically less susceptible to fluconazole with propensity to develop high-level resistance. In this report, we found the isolate to be resistant to itraconazole only. However, it had been previously

commented that despite *invitro* susceptibility to azoles and polyenes, these agents might not be that effective *in vivo*. Other therapeutic options should be tested in this context. Moreover, because of this association of drug resistance with this species of *Candida*, accurate species identification is very important not only for epidemiological significance, but also to provide guidance to physicians for effective treatment.

Elaboration of various virulence factors associated with colonizing and pathogenic strains of *Candida* have revealed them to be bearers of several traits responsible for their ability to easily survive and persist ininfection sites. The presence of phospholipase and proteinase activity was demonstrated in *C. auris* isolates in this study. Extracellular hydrolytic enzymes like phospholipases act as important virulence factors helping in adherence and invasion of host cells. Similarly, hemolysin production is followed by acquisition of iron that helps in hyphal extension and invasive disease thus leading to widespread infection [6].

Even after initial reports of isolation of *C. auris* from different body sites, its exact role in pathogenesis could not be ascertained in previous studies. Few researchers have in fact proved its pathogenic nature by performing histopathological study [11]. Instead, we studied the virulence traits of this isolate to reveal its pathogenic character.

*Candida spp*, most commonly *C. albicans* is a commensal of the vaginal flora in 20%-50% of women, but there has been an increasing trend in isolation of *C. glabrata, C. krusei, C. parapsilosis* from patients with symptomatic vulvovaginitis and their increasing resistance to commonly used antifungal drugs [5]. In this context, *C. auris* isolated in this patient and the association with drug resistance and virulence traits hints towards its increasing pathogenicity. Therefore, it should be realized that due to the diverse spectrum of pathogenic non-*albicans Candida spp* widens, laboratories face a real challenge in their prompt identification with available means. Improved molecular assays should be tried whenever conventional methods become insufficient.

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## Corresponding author


Ragini Tilak,
Department of Microbiology, Institute of Medical Sciences,
Banaras Hindu University,Varanasi-221005, India
Phone: +91 9415396353
Email: tilakragini28@gmail.com


**Conflict of interests:** No conflict of interests is declared.