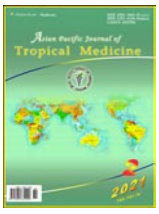

Case Report

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Biofilm-forming fluconazole-resistant *Candida auris* causing vulvovaginal candidiasis in an immunocompetent patient: A case report

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ABSTRACT

Rationale: *Candida auris* is a potential emerging pathogen among *Candida* and causes serious health threats globally.

Patient concerns: We reported a case of vulvovaginal candidiasis caused by *Candida auris*. A 26-year-old female presented with complaints of vaginal discharge, itching and low back pain.

Diagnosis: High vaginal swab culture yielded *Candida*. The strain was confirmed as *Candida auris* by amplification and sequencing the internal transcribed spacer region. Antifungal susceptibility testing revealed that the isolate was resistant to fluconazole, amphotericin B and clotrimazole and susceptible to ketoconazole and nystatin. The isolate also exhibited biofilm forming ability.

Interventions: Her symptoms did not subside with initial management with fluconazole and clotrimazole. Later, she was started on ketoconazole therapy. The patient responded well to ketoconazole.

Outcome and lessons: To the best of our knowledge, this is the first report about the presence of a drug resistant biofilm forming *Candida auris* strain isolated from a vaginal swab sample from Chennai area. Biofilm forming ability might contribute to its drug resistance. Nucleic acid analysis helps in rapid and accurate identification of such rare species.

KEYWORDS: *Candida auris*; Resistance; Biofilm; Scanning electron microscopy

1. Introduction

Candida (C.) auris is an emerging invasive pathogen reported from ear canal, blood, wound, respiratory tract infections, *etc*. *C. auris* was first isolated in 2009 in Japan from the external ear canal of a patient. Currently it exhibits resistance to fluconazole and shows variable susceptibility to antifungal drugs like amphotericin B and echinocandins[1]. Kumar *et al*. have reported a case of vulvovaginal candidiasis (VVC) caused by itraconazole resistant *C. auris*[2]. VVC is one of the frequent causes of vaginitis affecting women globally. Improper use of antifungal agents like incorrect dosage, prolonged hospitalization, poor immune status are some other factors for emergence of unusual species like *C. auris*. Due to its resistance to other conventional drugs, the pathogen has turned out to be difficult to be treated because of its altered virulence mechanism which includes drug efflux and dimorphism characteristics. The increasing occurrence as well as the varying antifungal drug susceptibility profiles of *C. auris* emphasizes the importance of identifying *Candida* to the species level in diagnostic laboratories. Understanding the antifungal susceptibility profile of various *Candida* species helps in developing appropriate protocols for empirical antifungal therapy in emergency cases. There is a huge need for new antifungals to treat the resistant strains.

In the present study, we report a case of 26-year-old female with vulvovaginal candidiasis caused due to *C. auris*, identified by both phenotypic and molecular methods. We also sought to examine the biofilm forming potential of the isolate along with their antifungal susceptibility profile. A written consent has been obtained from the patient for publication and the study has been approved by the institutional ethical committee (Ref. No. 002/ SBMC/ IHEC/2016/189).

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2. Case report

A 26-year-old female presented to the Department of Gynaecology, Sree Balaji Medical College and hospital, Chennai with complaints of vaginal discharge, low back pain and itching sensation in the vaginal area. Two high vaginal swab samples were collected and processed for bacterial and fungal culture. Direct microscopy showed the presence of ovoid budding yeast cells. The sample was inoculated in Sabouraud Dextrose Agar (SDA) (Hi Media, India) for culturing fungi and on Nutrient agar, Blood agar and Mac Conkey agar plates (Hi Media, India) for culturing bacteria. The inoculated culture plates were incubated at 37 ℃ for 48 h.

After 48 h of incubation, bacterial culture was negative. SDA plate showed white to cream coloured smooth colonies. Gram staining showed Gram positive budding yeast cells. The colonies were sub-cultured on CHROM agar (Hi Media, India) for species identification. No characteristic colour was produced by the isolate in CHROM agar plate. Sugar assimilation and fermentation tests were indecisive. The antifungal susceptibility testing was performed as per manufacturer's instructions using disc diffusion test as per Clinical and Laboratory Standards Institute M44A document[3], using fluconazole (25 μg), itraconazole (10 μg), nystatin (100 Unit), clotrimazole (10 μg), ketoconazole (10 μg) and amphotericin B (100 Unit) (Hi Media, Mumbai, India). The yeast cell suspension was prepared by inoculating 5 isolated yeast colonies from SDA plate in 5 mL of sterile 0.85% saline and turbidity adjusted to 0.5 McFarland standard. The isolate was found to be resistant to fluconazole, amphotericin B and clotrimazole. It showed a dose dependent susceptibility to itraconazole and was susceptible to ketoconazole and nystatin.

The strain was further processed for molecular identification and examined for its ability to form biofilms. The fungal DNA was extracted and the Internal Transcribed Spacer (ITS) region of *18S* rRNA was amplified using the primers ITS1 (forward) 3'TCCGTAGGTGAACCTGCGG-5' and ITS4 (reverse) 5'TCCTCCGCTTATTGATATGC-3'[4]. PCR products were purified and sequencing was carried out by the Dideoxy chain termination method (Eurofins, Bangalore). The resulting sequence was submitted in GenBank under the accession number MK108049.

The biofilm forming ability of *C. auris* was assessed by the classical ring test[5]. A total of 1 mL of yeast peptone dextrose broth (Hi Media, India) was supplemented with 20 μL of yeast cell suspension and incubated statically at 37 ℃ for 24 h. The planktonic cells were removed and *C. auris* formed biofilms as a ring on the walls of the test tube which was stained and observed using crystal violet (Hi Media, India) (Figure 1A). Preformed or mature biofilms of *C. auris* were observed by adding 100 μL of *C. auris* cells to 1 mL of yeast peptone dextrose broth in a 24-well polystyrene plate (Tarsons, India). The plate was incubated at 37 ℃ for 48 h without agitation. After crystal violet staining, *C. auris* formed a thick mat, indicating a well-developed biofilm which was adhered to the bottom and sides of the microtiter well (Figure 1B). The slide with the biofilm cells was dried and examined under Scanning Electron Microscopy. SEM helped to visualise the biofilms formed by *C. auris* on glass slides. The *C. auris* strain formed a biofilm with a thick aggregation of cells and the budding yeast cells embedded in the slime layer were also observed in scanning electron microscopy VEGA3 TESCAN (China) (Figure 1C).

The patient was initially started on empirical treatment of single dose of oral fluconazole 150 mg and intravaginal clotrimazole 1% cream 5 g for 7 days. However, the patient did not show any improvement clinically. With the antifungal susceptibility report of the patient, the patient was started on oral ketoconazole 200 mg twice daily for 2 weeks. The patient responded well to ketoconazole. She improved and her symptoms got relieved. The patient was asymptomatic in her follow up visit after 2 weeks.

3. Discussion

C. auris has gained importance in recent years due to the emergence of drug resistant strains in this species[1]. *C. auris* isolate from non-blood source in a study by Larkin *et al*, showed decreased susceptibility to fluconazole when compared to other *C. auris* isolates[6]. In the present study, the isolate was resistant to fluconazole and amphotericin B in concordance to previous reports[6,7]. *C. auris* strains occasionally get misidentified because the current culture based diagnostic methods fail to distinguish them from other closely related species[7]. This factor stresses the need to employ molecular identification tools for appropriate diagnosis of

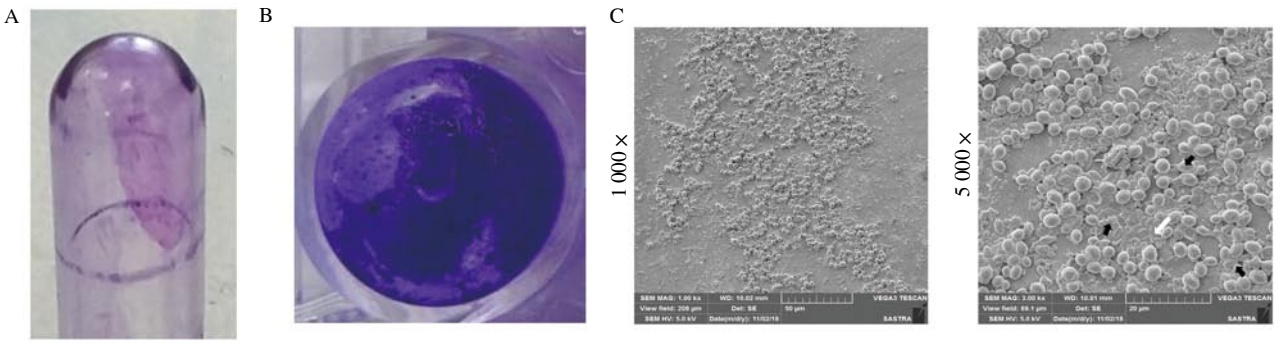

Figure 1. Biofilm formation by *Candida auris* which were collected from a 26-year-old female with vulvovaginal candidiasis. (A) Young biofilm of *Candida auris* formed as a ring at the air liquid interface. (B) A thick mat of matured biofilm formed at the bottom of polysterene plate well. (C) Scanning electron microscopy analysis of biofilm.

Candidal infections rather than rely only on culture based methods.

C. auris has been reported causing candidaemia and wound infections[1,8]. *C. auris* has also been recently reported from an immunocompetent ICU patient[9]. The increasing reports of *C. auris* isolated from various clinical specimens clearly indicate the ability of the pathogen to colonise, invade and cause various diseases. Biofilm formation by *C. auris* is considered as one of the virulence factors attributing to its drug resistance[6]. It was interesting to notice that the budding yeast cells were embedded in a thick slime layer. Slime is an important component of the exopolysaccharide layer of biofilms formed by most of the *Candida* strains. This sequesters antifungal drugs, preventing them from reaching their cellular targets which leads to drug resistance. Slime also aids in the anchorage of *C. auris* cells on inert surfaces, making it capable to persist on nosocomial surfaces. Recent reports suggest that *C. auris* has successfully spread in nosocomial environments and was even detected among healthcare staff indicating the efficient human to human transmission of this strain[10]. The ability of *C. auris* to survive in biofilm-form in patients is considered to be an important factor of its resistance to systemic antifungals and is linked to increased morbidity and mortality[10]. *Candida* strains have evolved effective resistance mechanisms for the commonly encountered drugs. Resistance mechanisms include modification in the target enzyme which results in point mutation in *ERG11* gene, which is the target for azole class of antifungals. This may be due to different affinities of azoles that have different structures leading to differential activity. Future treatment regimens should target on destroying the biofilms of *Candida* sp. with antibiofilm agents in combinations with anti-fungal agents. Currently, very little is known about the virulence factors, transmission and mechanisms of drug resistance in *C. auris*. Species identification is critical for epidemiology, appropriate management as well as for implementation of infection control practices targeting *C. auris* biofilms.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Authors' contributions

L.K. collected samples and isolated the strain. J.S. performed the biofilm analysis and imaging. L.K., C.S. and P.N. contributed to the final version of the manuscript. L.K. and P.N. supervised the project.

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