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Tip to correct the variation position error in applying long-read high-throughput sequencing technology for fungal identification

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Presently, long-read high-throughput sequencing technology has been started applying in the medical mycology field instead of common Sanger sequencing. With its capability to sequence nucleic acid among the large region compared to Sanger sequencing, this technology can detect and analyze both inter- and intra-species sequence variation in internal transcribed spacer (ITS), a fungal house-keeping region, resulting in high accurate fungal identification. However, to set up the long-read high-throughput sequencing technology for fungal identification is quite challenging because of several factors related to the accuracy including the variation position error. So, in this study, we summarize the tip to correct the variation position error in applying long-read high-throughput sequencing technology for fungal identification that we have learnt in the preliminary laboratory setting using the 8 clinical isolates of yeast: *Candida albicans* ($n = 2$), *C. tropicalis* ($n = 1$), *C. glabrata* ($n = 1$), *Trichosporon* asahii ($n = 2$), *Pichia kudriavzevii* ($n = 1$), *Cryptococcus neoformans* var. *grubii* ($n = 1$). Based on the recruited strains, we found that self-assembling reference sequence generated from raw data of reading by using an auto-program named Canu causes the size-inflated sequence, a larger size calculated as $22.83 \pm 7.56\%$ than it should be, resulting in the shift of variation position. This error can be corrected by the alignment process of the reference sequence with the known sequence, both size and position, prior to doing the raw read alignment. The advantage of this process could correct not only for position shifting caused by the analysis process but also the random error generated from nanopore system. To validate this correction protocol, more samples are needed for further study.

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Detection of erg11 gene mutation in fluconazole resistant *Candida albicans* isolates

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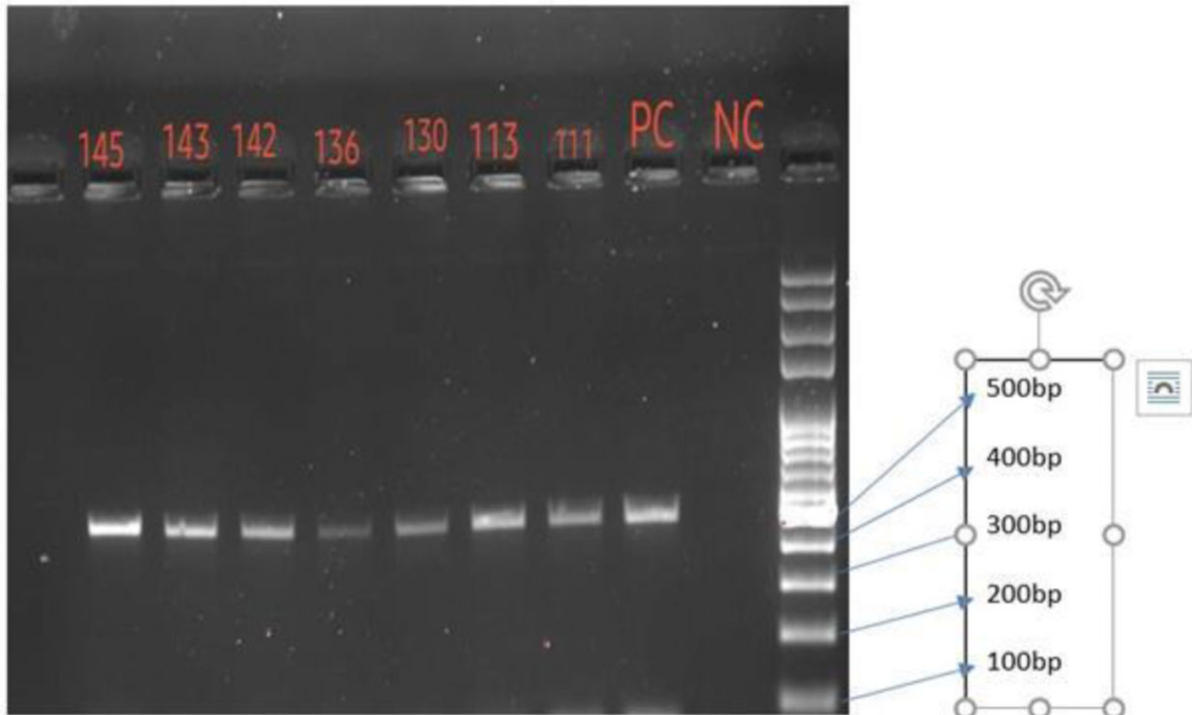
Objective: To determine the profile of fluconazole-resistant *Candida* isolates (FRCS) and the proportion of erg11 gene mutation in fluconazole-resistant *C. albicans* (FRCA) isolates

Methods: After getting approval from ethics the study was conducted on 150 isolates of FRCS obtained from patients admitted to our tertiary care hospital from September 2019 to December 2021. All the isolates were revived onto Sabouraud dextrose agar (SDA) and identified by Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS). Fluconazole-resistance was detected by VITEK 2 automated system. Deoxyribonucleic acid (DNA) of FRCA isolates were extracted by QIAamp DNA Mini kit (Qiagen-51 304, Hilden, Germany) as per manufacturer's instruction. A single primer pair targeting the nucleotide sequence from 1067 to 1576 bp region of erg11 gene was used for amplification as per Xu et al. with minor modification and 500 bp polymerase chain reaction (PCR) products were observed by gel electrophoresis. A positive clinical isolate of FRCA was used as a positive control. The PCR products were purified and subjected to Sanger sequencing with an ABI PRISM DNA analyzer (Applied Biosystems) and mutations were detected by using Mega (version 7) software by comparing with the published GenBank sequence AF153844.1 of *C. albicans* strain ATCC 28516. Categorical variables were expressed in percentages and continuous variables were expressed in mean (average).

Results: Out of the total of 150 FRCS, the most common fluconazole-resistant agent of candidiasis was *C. auris*, which was isolated from 60 (40%) cases followed by 24 (16%) isolates of *C. glabrata*, 19 (12.7%) isolates of *C. krusei*, 18 (12%) isolates of *C. parapsilosis*, 17 (11.3%) isolates of *C. albicans*, 10 (6.7%) isolates of *C. tropicalis*, and only two (1.3%) isolates of *C. guilliermondii*. Among these 17 FRCA isolates, 9 isolates were obtained from genital swabs, 5 from blood, and a single isolate each from the perianal swab, ear swab, and sputum. A total of 12 (70.6%) out of 17 FRCA isolates yielded 500 bp region in erg11 gene. Gel electrophoresis image of PCR products of erg11 gene is depicted in Figure 1. Sequencing of these products detected a single missense mutation A1309G (V437I) in one FRCA isolate. Most of the FRCA isolates had an average of 4 silent mutations (observed in 6 isolates) and a single isolate had 6 silent mutations. A total of 8 different silent mutations were observed among FRCA isolates T1296C (83.3%), C1203T (66.7%), A1440G (66.7%), C1302T (50%), T1470C (50%), T1140C (25%), T1110C (16.7%), and T1284C (8.3%).

Conclusion: *C. auris* was the most common fluconazole resistant isolate observed, followed by *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. albicans*, *C. tropicalis*, and *C. guilliermondii*. There were many silent mutations observed in erg11 gene of FRCA isolates and detected only a single missense mutation (V437I). Fluconazole-resistant in FRCA isolates may be due to mechanisms other than the studied one in our region.



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