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Identification of *ERG11* gene mutations in fluconazole-resistant *Candida albicans* isolates from superficial candidiasis

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ABSTRACT

Aims: Superficial candidiasis is a skin infection caused by an increasing pathogenic fungus *Candida albicans* population. Data from various public health education hospitals in Indonesia show a high prevalence of superficial candidiasis. Fluconazole is one of the main options for the treatment of superficial candidiasis, but due to its uncontrolled use, it can induce resistance in *C. albicans* isolates. Most of the molecular mechanisms of *C. albicans* resistance to fluconazole are caused by mutations in the *ERG11* gene. This study was aimed to identify mutations in the *ERG11* gene in fluconazole-resistant *C. albicans* isolates from superficial candidiasis.
Methodology and results: The identification of *ERG11* gene mutations was carried out based on the results of DNA sequencing. Results of the amino acid alignment showed that the impact of the mutation was eight silent mutation codons due to transversion substitution (F105F, L220L, V332V, K342K, L370L, Y401Y, A432A, and A434A). One codon had a missense mutation due to transition substitution (E266D), and one codon had a single nucleotide polymorphism (SNP), A or T substitution, which could produce two different types of amino acids, namely aspartic acid and glutamic acid (D116E).
Conclusion, significance and impact of study: Amino acid substitutions E266D and D116E were identified in this study but did not contribute to the resistance mechanism of *C. albicans* to fluconazole. These results could serve as a source of information to be used in treating candidiasis cases because, indirectly, the results of this study explain that there are other mechanisms related to the resistance of *C. albicans* isolates to superficial candidiasis.

Keywords: Candida albicans, *ERG11*, *DNA*, fluconazole-resistance, mutation

INTRODUCTION

Superficial candidiasis is an acute or sub-acute mycosis infection caused by an increase in the population of fungi in the genus *Candida* on the skin or mucosal layers (Nurdin *et al*., 2021). Common causes of superficial candidiasis are *Candida albicans* (48%), followed by *Candida krusei* (16.1%), *Candida glabrata* (13.5%), *Candida kefyr* (7.4%), *Candida parapsilosis* (4.8%), *Candida tropicalis* (1.7%), and other *Candida* species (8.5%) (Dufresne *et al*., 2014).

Superficial mycosis caused by *C. albicans* is estimated to affect around 20-25% of the world's population and is one of the most common forms of infection in humans (Marsaoly *et al.*, 2014). Cases of skin disorders in China caused by *C. albicans* infection in 2017 were reported to rank third at 14% of fungal infections of the skin, and in Singapore, cases of superficial candidiasis ranked fourth (Angrraeni *et al.*, 2019). Cases of candidiasis in Indonesia itself are still common. Based on research data at Dr. Moewardi Hospital Surakarta for the period January 2016-December 2019, there were 177 patients infected with superficial candidiasis (Nurrachmat and Fiqnasyani, 2022). The same case was also found at Undata General Hospital from 2013-2021; superficial candidiasis infection was found in 284 cases (18.02%) (Sofyan and Hikmah Buchair, 2022). Skin and genital clinic dr. Soedono Hospital Madiun also reported that during January-December 2021, 80 new cases of superficial mycosis were found, with a diagnosis of 21% of them being a superficial candidiasis infection (Widhiastuti *et al.*, 2023).

Treatment for candidiasis infection can be performed with antifungal therapy. Azole antifungals such as fluconazole (FCA), itraconazole (ITR), and voriconazole (VRC) were identified as the primary clinical treatments

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for *C. albicans* infection (Feng *et al*., 2017). Fluconazole and itraconazole are first-generation triazoles that are used in Asia due to their availability. Voriconazole, a second-generation triazole, was initially introduced to the market with a broad-spectrum ability to combat fungal diseases; they are similar in effectiveness against *Candida* spp. (Partha *et al*., 2022). Fluconazole is 97.9% effective for *C. albicans* (Pfaller and Diekema, 2007).

Clinical treatment failure of candidiasis has been reported in several studies due to the resistance of *Candida albicans* to antifungal agents (Oliveira *et al*., 2021). Uncontrolled use of antifungals in clinical practice causes candidiasis infection and induces resistant isolates (Feng *et al*., 2017). *Candida albicans* resistance has been reported in several previous studies, with the highest resistance rate to azole antifungals such as fluconazole (Gubbins and Anaissie, 2009). Research on the susceptibility of fluconazole to *C. albicans* in 2014-2022 revealed that 35% of *C. albicans* isolates were resistant to fluconazole (Mohammadi and Shalavi, 2014; Dovo *et al*., 2022).

Most of the molecular mechanisms of *C. albicans* resistance to antifungal agents are caused by gene mutations (Candrasari, 2014). The impact of mutations, specifically the over-expression of membrane transporters, altered import of cholesterol, altered ergosterol biosynthesis, and altered genome plasticity (Bhattacharya *et al*., 2020). The *ERG11* gene is a frequently mutated gene in *C. albicans* and is the leading cause of resistance in Candida cells to antifungals (Flowers *et al*., 2015).

The *ERG11* gene encodes lanosterol 14-alpha-demethylase, a vital enzyme in the ergosterol biosynthesis pathway of *Candida* species and other organisms. Ergosterol is essential for fluidity and stability of fungal cell membranes. Lanosterol 14-alpha-demethylase catalyzes the demethylation of lanosterol, a precursor in ergosterol synthesis, leading to ergosterol production. The enzyme's activity is crucial for fungal cell viability and function (Song *et al*., 2004). Mutation in *ERG11* may lead to inhibition of lanosterol 14-alpha-demethylase disrupts ergosterol production, alter the fungal cell membrane and increase permeability, ultimately resulting in cell death.

With increasing fungal infections and limited treatment options and diagnostic methods available, antifungal resistance may become a serious problem in the future (Canuto and Rodero, 2002). An accurate diagnosis of *C. albicans* resistance to fluconazole due to mutations in the ERG11 can be made by analysis based on molecular polymerase chain reaction (PCR) diagnostics followed by DNA sequencing to determine the genetic diversity of *C. albicans*, which illustrates the nature of *C. albicans* resistance to fluconazole (Kordalewska and Perlin, 2019).

MATERIALS AND METHODS

Sample collection

The research was conducted at the Laboratory of Microbiology and Molecular Biology, Faculty of Nursing and Health, Universitas Muhammadiyah Semarang. The samples used in this study were obtained from dermatomycosis wound surface swabs from six patients at the Dermatology and Venereology Polyclinic at Sunan Kalijaga Demak Hospital, Indonesia, with a diagnosis of superficial mycosis. The characteristics of the wound selected as the sample were the appearance of hard and very itchy vesicles accompanied by scaling or exfoliation of the skin (Madani and Harahap, 2000).

The patients were confirmed regarding the method of treatment and the type of antifungal used, both orally and superficially, to ascertain whether there was a suspicion of resistant isolates. This study was approved by the Ethical Committee of the hospital and Faculty of Medicine, University Sultan Agung Semarang, Indonesia (No. 276/VII/2023/Komisi Bioetik), and all patients gave their written consent before enrolling in the study.

Isolation and identification

Samples were identified using streak plate culture on Sabouraud Dextrose Agar (SDA) medium added with tetracycline antibiotics and incubated at 37 °C for 24-48 h. Microscopic identification of *C. albicans* was carried out by simple staining (crystal violet). Then proceed with the Germ Tube Test (GTT) (Jawetz *et al*., 1996).

Antifungal susceptibility test in *C. albicans*

The *C. albicans* colonies that grew on SDA medium had the characteristics of being round, creamy in color, smooth consistency, and smelled like yeast. They were re-cultured on SDA medium that had been added with fluconazole (100 mg/50 mL) (Jain *et al*., 2001). Incubation: 37 °C for 24-48 h. The growing colonies were observed and taken to the DNA extraction step because it was suspected that *C. albicans* was resistant to fluconazole.

DNA extraction

Fluconazole Resistant *C. albicans* isolates from SDA plates were cultured in Sabouraud Dextrose Broth (SDB) medium and incubated at 37 °C until the medium turned cloudy to allow *C. albicans* to multiply. Isolation of *C. albicans* DNA using a solid base method with the gSYNCTM DNA Extraction Kit (Geneaid) used column filter. Procedures according to the kit protocol. Isolate DNA results were measured the concentration and purity using a Nano Pro Maestro GEN spectrophotometer.

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PCR amplification

The *ERG11* gene was detected by conventional PCR using specific primers. The primers used to detect sequences in the exon of *ERG11* that appear to be related to resistance due to *C. albicans* exposure to fluconazole (Rosana *et al*., 2015). The primer sequence and PCR program can be seen in Tables 1 and 2. Electrophoresis on 2% agarose gel (prepared in 1× tris base-borate EDTA solution) at 70 V for 90 min was used to separate the amplicon PCR products.

Table 1: PCR composition.

No.	Component	Volume (µL)	Final Concentration
1	PowerPol 2× PCR	12.5	1×
2	DNA Template	2	<250 ng
3	Nuclease-free Water	6.5	N/A
4	Primer ERG11-F [20] 5'-ATGGCTATTGTTGAAA CTG-3'	2	0.1-1 µM
5	Primer ERG11-R [20] 5'-TTAAAACATACAAGTTT CTCTT-3'	2	0.1-1 µM
	Total volume	25	

Table 2: PCR program.

No.	Step	Temp	Time	Cycles
1	Pre-denaturation	94 °C	3 min	1
2	Denaturation	94 °C	60 sec	
3	Annealing	49 °C	60 sec	40
4	Extension	72 °C	60 sec	
5	Pos-extension	72 °C	3 min	1
6	Hold	4 °C	~	1

DNA Sequencing

PCR products were sent to third-party company for DNA sequencing by the Sanger method. Results of DNA sequences will be assembled using the DNA Baser v5 program to get the contig sequence and alignment using the online program Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov/) in comparison with the nucleotide sequence of *C. albicans* strain SC5314 (XP_716761.1).

RESULTS

Identification and antifungal susceptibility testing

The culture results on 6 samples were obtained from a purposive sampling of dermatomycosis wound swabs (CA1-CA6) and showed that six isolates produced colonies with same characteristics, convex-rounded morphology, cream colour, characteristic yeast-like odour, and smooth consistency, according to the morphological characteristics of *C. albicans* colonies (Figure 1).

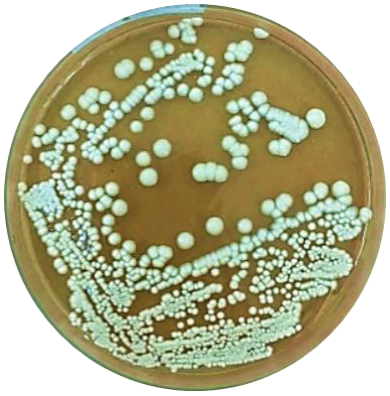

Figure 1: Colony results of fluconazole resistant *Candida albicans* on SDA media + fluconazol.

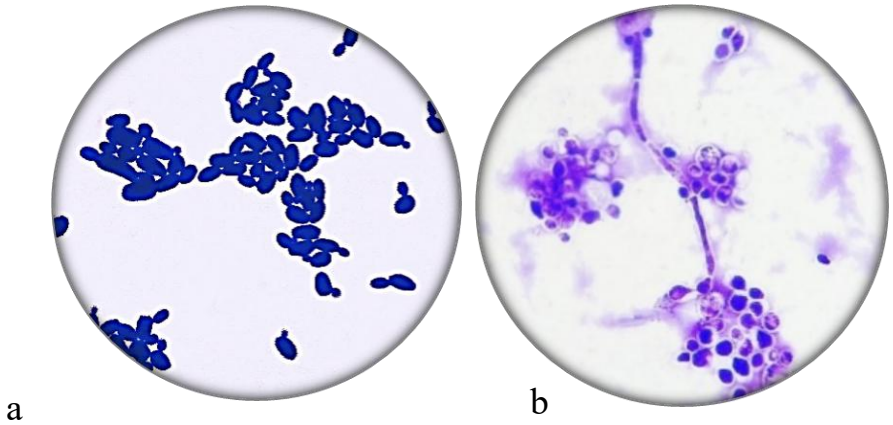

Figure 2: Gram A staining results of *C. albicans* (a) and GTT test (b).

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The results of crystal violet staining obtained six isolates showing oval-shaped cell morphology, purple colour, and solitary arrangement. While the GTT test results of six isolates showed that the blastospores that germinated produced pseudo-hyphae and chlamydospores, indicating that *C. albicans* was pathogenic (Figure 2) (Munin *et al.*, 2007; Rusu *et al.*, 2014).

The results of the culture sensitivity test of *C. albicans* in fluconazole SDA medium showed that one sample was sensitive to fluconazole (CA1), and 5 samples were resistant to fluconazole (CA2, CA3, CA4, CA5, and CA6). Resistant *Candida* is characterized by the growth of colonies on the media after the incubation period is complete.

DNA extraction

DNA concentration and purity from samples show good results (Table 3.). The absorbance of λ 260 nm/ 280 nm is in the range of 1,8-2,0 and the measured concentration is in the range of more than 50 ng/μL.

Table 3: DNA concentration and purity.

No.	Sample Code	DNA Concentration (ng/μL)	Absorbance λ 260 nm/280 nm
1	CA2	94,39	1,935
2	CA3	93,15	1,990
3	CA4	72,48	1,954
4	CA5	64,66	1,972
5	CA6	55,84	1,883

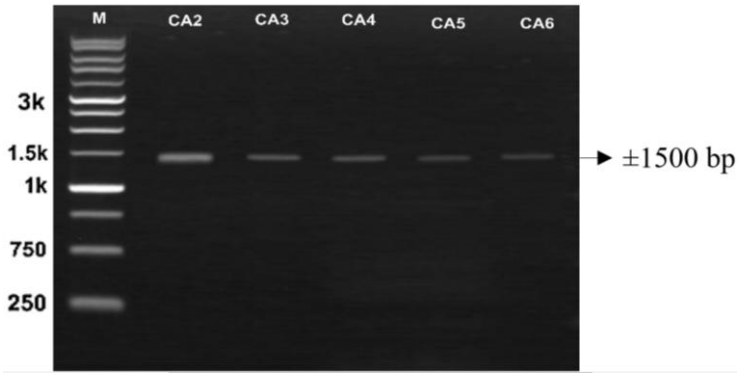

Figure 3: Electrophoresis result. Lane M: Marker 1 kb. CA2-CA6 was *C. albicans* isolates.

DNA amplification

The results of the amplification of 5 isolates CA2-CA6 (fluconazole resistant) in 2% agarose gel produced a single band close to the DNA marker with a molecular length of ± 1500 bp (Figure 3). The 5 samples that produce DNA fragment was selected to proceed to the DNA sequencing process. CA2 was used as representative sample for DNA sequencing.

***ERG11* gene mutation analysis**

The sequencing results were assembled using a DNA baser into a complete sequence (consensus or contig sequence) with a base length of ± 1500 bp. The contig was aligned with the available sequence data in the database. The alignment results showed a contig similarity with the sequence of partial mRNA sterol 14-a demethylase (*ERG11*) *C. albicans* strain SC5314 (XM_711668.2) with a percent identity of 98.91%. Contig targets start from DNA sequences 51-1534. The SC5314 strain was chosen as a reference because this strain is a *C. albicans* strain that is sensitive to fluconazole and is often used as a laboratory standard wild-type strain (Muzzey *et al.*, 2013). The results of the sequence contigs that had assembled and aligned the CA2 sequence show discrepancies in some parts of the sequence due to the mutation process. The sequencing result found 10 nucleotides that have substitution mutations (Figure 4).

Mutation causes changes in the sequence of amino acids in proteins, resulting in reductions, alterations, or loss of enzyme function. As a result of the alignment of the amino acid sequence of the CA2 sample with the sequence of *C. albicans* strain SC5314 (XP_716761.1), we found 10 sites of mutation as a result of a substitution mutation (Figure 5).

Several mutations were detected in the sequencing results, including eight silent mutations in F105F, L220L, V332V, K342K, L370L, Y401Y, A432A and A434A. One missense mutation in E266D and one Single Nucleotide Polymorphism (SNP) substitution mutation in D116E. The mapping results can be seen in Table 4.

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Figure 4: The results of the *ERG11* target contig. The yellow colour indicates the location of the substitution mutation. The sign (W) indicates a T or A substitution.

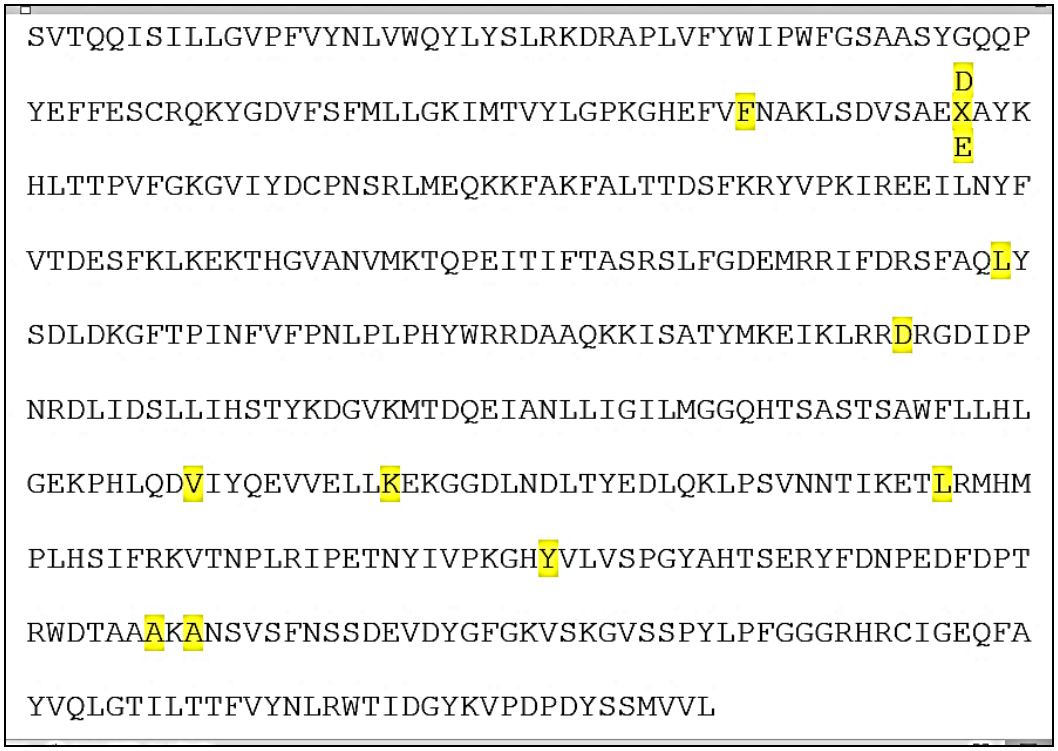

Figure 5: ERG11 amino acid sequence. The yellow colour indicates the location of the amino acid mutation. X letter in picture was SNP with alternative coding D (aspartic acid) or E (glutamic acid).

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Table 4: Mapping results of nucleotide bases and amino acids of the *ERG11.*

Mutation	No.	Amino Acid	Substitution	Nucleotides	Description
Silent Mutation	1	F105F	Transition substitution	315 T>C	*Phenylalanine* at codon 105 is not replaced
	2	L220L	Transition substitution	658 C>T	*Leucine* at codon 220 is not replaced
	3	V332V	Transition substitution	996 T>C	*Valine* at codon 332 is not replaced
	4	K342K	Transition substitution	1026 A>G	*Lysine* at codon 342 is not replaced
	5	L370L	Transition substitution	1110 C>T	*Leucine* at codon 370 is not replaced
	6	Y401Y	Transition substitution	1203 T>C	*Tyrosine* at codon 401 is not replaced
	7	A432A	Transition substitution	1296 C>T	*Alanine* at codon 432 is not replaced
	8	A434A	Transition substitution	1302 T>C	*Alanine* at codon 434 is not replaced
Missense Mutation	9	E266D	Substitution transversion	798 A>C	*Glutamic acid* at codon 266 is replaced by *aspartic acid.* Reported by: Marichal *et al*. (1999) and Goldman *et al*. (2004).
SNP-substitution	10	D116E	SNP W (Substitution T or A)	348 T>T 348 T>A	At codon 116, two amino acids are formed, namely *aspartic acid* and *glutamic acid.* Reported by: Perea *et al*. (2001) and Chau *et al*. (2004).

DISCUSSION

Candida albicans is a pathogenic *Candida* species often found to cause superficial and systemic infections (Muzzey *et al*., 2013). Under certain conditions, *C. albicans* can overgrowth and become invasive, causing systemic disease in patients with weak immune systems (Jawetz *et al*., 1996; Marichal *et al*., 1999). In the pathogenic state, *C. albicans* is found in the form of pseudo-hyphae and hyphae, whereas in the commensal state, it is found in the form of blastospores (a spherical, oval, or elliptical shape with a size of 3-15 µm) (Goldman *et al*., 2004).

Patients with dermatomycosis wounds selected as subjects were confirmed to work as farmers who go to the rice fields daily. The surface of the feet, which is always wet and moist, it facilitates the local invasion of *C. albicans*. The differentiation of *C. albicans* between blastospores, pseudohyphae, and hyphae is a form of adaptation of *C. albicans* to the surrounding environment. Several factors, such as temperature, pH, nutrition, and a humid environment, can affect the morphology and virulence factors of *C. albicans* (Perea *et al*., 2001).

Candidiasis can be treated in two ways, which were superficial or locally and systemically (Marsaoly *et al*., 2014). In the treatment of superficial candidiasis or systemic fluconazole antifungals are used to target the ergosterol biosynthetic pathway (Chau *et al*., 2004). Treatment with antifungals, especially those with a broad spectrum, in high doses for a long time can increase the colonization of Candida, which originally lived as saprophytes and then changed its nature to become a pathogen (Coleman *et al*., 2012). This condition is caused by the use of antifungals that can suppress the growth of normal flora on the surface of the skin and cause *Candida* to grow more fertile. Since 1990, cases of fluconazole treatment failure due to the development of resistance in the pathogenic fungus *C. albicans* have been reported (Pratiwi, 2008).

Fluconazole targets the enzyme lanosterol 14-alpha-demethylase, encoded by the *ERG11* gene in fungi, including *Candida* species. This enzyme plays a key role in the ergosterol biosynthetic pathway, essential for the production of ergosterol, a vital component of the fungal cell membrane. Lanosterol 14-alpha-demethylase catalyzes the demethylation of lanosterol, a precursor in this pathway, which is crucial for converting it into ergosterol. Fluconazole, an azole antifungal, binds to the enzyme's active site, forming a complex with the heme group and inhibiting its activity. This binding prevents the demethylation of lanosterol, disrupting the conversion to ergosterol. Depletion of ergosterol, critical for the fungal cell membrane, leads to changes in membrane composition and structure, compromising fungal cell viability.

An antifungal sensitivity test was carried out to distinguish sensitive and resistant *C. albicans* isolates to fluconazole. Culture results on SDA fluconazole 100 mg/50 mL showed 1 fluconazole-sensitive isolate and 5 fluconazole-resistant isolates. The results of this sensitivity test are consistent with previous studies. A 2014 study in Iran found 96.7% of cases of *Candida* resistance to fluconazole, while in 2016 in India it was found in 74.1% of cases, and a 2022 study in Africa reported a 35.0% incidence of treatment failure due to *Candida* resistance to fluconazole (Mohammadi and Shalavi, 2014; Mane *et al*., 2016; Dovo *et al*., 2022).

Several molecular mechanisms explaining the resistance of *C. albicans* to azole antifungals have been reported. One such mechanism is a mutation in the *ERG11* gene, which encodes the target enzyme, lanosterol 14 a-demethylase (Feng *et al*., 2017). This enzyme plays an important role in yeast ergosterol biosynthesis, which is responsible for the conversion of lanosterol to ergosterol (Oliveira *et al*., 2021). The results of biosynthesis in the form of ergosterol will be placed in the fungal cell membrane, which functions as a system of rigidity, membrane stability, and defence against physical stress (Pratiwi, 2008).

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Based on the results of the PCR amplification of the *ERG11* gene, DNA bands were formed in 5 samples with a size of around 1500 bp, which were CA2-CA6 samples (resistant isolates), while CA1 samples (sensitive isolates) did not form bands. The susceptible isolates did not produce DNA fragments, probably because the *ERG11* gene of the susceptible isolates was not amplified. *ERG11,* which encodes the enzyme lanosterol 14 α-demethylase, which plays a role in the rigidity and stability of cell membranes, was inhibited by the addition of the antifungal fluconazole (100 mg/50 mL) during the sensitivity test. The *ERG11* gene, which is affected by azoles, induces ergosterol deficiency in the fungal cell membrane, and *C. albicans* cells become sensitive (Candrasari, 2014).

In the two aligned sequences between sample and reference, a mismatch was found due to gene mutations, transition substitution and transversion substitution. Based on the alignment results, there were 8 base points where transition substitution mutations occurred, 1 base point of transversion substitution, and 1 base point where the mismatch occurred due to a Single Nucleotide Polymorphism (SNP).

SNPs are the most frequently observed differences at the nucleotide level in diploid organisms. *Candida albicans* is a diploid fungus that reproduces by clonal mitotic division with a high level of heterozygosity. SNPs are found in the coding regions of genes or in intergenic regions. In the codon region, SNPs can change the function and structure of the protein they encode, for example, proteins involved in drug metabolism (Forche *et al*., 2004; Morais *et al*., 2020).

The results of the mapping of nucleotide bases and amino acids revealed the impact of the mutation, namely 8 silent mutation codons due to transversion substitution (F105F, L220L, V332V, K342K, L370L, Y401Y, A432A, and A434A). In addition, it was also found that one codon had a missense mutation due to transition substitution (E266D), and one codon had a SNP (*adenine* or *thymine* substitution), which could produce two different types of amino acids, namely aspartic acid and glutamic acid (D116E) (Table 4).

Based on previous research in 2013, it detected 19 nucleotide substitutions in the *ERG11* gene, four of which were D116E, K128T, V159I, and E266D. Then in 2009 study found amino acid substitutions in the *ERG11* gene, which are D116E, E266D, K128T, V437I, and V488I. Whereas in 1997, a study identified a number of point mutations that were only present in resistant isolates, which were F105L, E266D, K287R, G448G, G450E, G464S, and V488I (Löffler *et al*., 1997; Wang *et al*., 2009; Strzelczyk *et al*., 2013).

Indonesia is a country that has thousands of islands, resulting in variations in the *ERG11* gene mutation in each region. Variation in mutations in the *ERG11* gene in superficial candidiasis isolates obtained from Sunan Kalijaga Demak Hospital, Central Java, Indonesia, identified 3 mutations that are the same as studies by Löffler *et al*. (1997); Wang *et al*. (2009); and Strzelczyk *et al*. (2013), which were mutations in codons D116E and E266D. Both amino acid changes have been reported previously: D116E (Perea *et al*., 2001; Chau *et al*., 2004) and E266D (Marichal *et al*., 1999; Goldman *et al*., 2004), ERG11 amino acid substitution at E266D was most frequently found in multi-resistant *C. albicans* isolates (fluconazole, itraconazole, and voriconazole) (Rosana *et al*., 2015). However, many other studies have also stated that substitution of E266D with D116E is also often found in azole-sensitive strains, so there is a possibility that these mutations do not contribute to the decrease in fluconazole activity (Löffler *et al*., 1997. Sanglard *et al*., 1998; Marichal *et al*., 1999; Perea *et al*., 2001).

Research on the detection and analysis of ERG11 mutations that cause resistance of *C. albicans* superficial candidiasis isolates to fluconazole in Indonesia is rarely carried out, so this research will be instrumental in handling cases of superficial candidiasis infection. Analysis of *C. albicans* genetic variation will provide key information in choosing the type of treatment in patients with superficial candidiasis.

CONCLUSION

In this study, a potential amino acid change mutation was found in the *ERG11* gene, which suggested causes resistance in *C. albicans* to fluconazole, namely E266D and D116E. Previous research suggests that this substitution can be found even in susceptible isolates. Perhaps changes in amino acids in the *ERG11* gene at other positions have more impact than those targeted in this study. This suggests that the mutations that have been found are not sufficient to characterize the mechanism of resistance, and most likely there are demands for other resistance mechanisms that have not been elucidated and could provide a better correlation with drug resistance.

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AUTHORS' CONTRIBUTION

The authors declare that all listed authors have contributed equally to the conceptualization, formal analysis, methodology, review preparation, and edition of the present research. All authors have read and approved the submitted final manuscript.

CONFLICT OF INTEREST

In relation to this article, we declare no conflict of interest.

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REFERENCES

Angrraeni, Y. D., Mumpuni, W. D., Sutanto, G. and Wijayanti, R. (2019). Halal cosmeceutical: Kuteks wudlu friendly dan terapi dermatomikosis dari ekstrak pacar air (*Impatiens balsamina* L). *Media Farmasi Indonesia* **14(2), 1540-1545.**

Bhattacharya, S., Sae-Tia S. and Fries B. C. (2020). Candidiasis and mechanisms of antifungal resistance. *Antibiotics* (Basel) **9(6), 312.**

Candrasari, D. S. (2014). Kajian molekuler resistensi *Candida albicans* terhadap antifungi. *Jurnal Farmasi Sains Dan Komunitas (Journal of Pharmaceutical Sciences and Community)* **11(1) 43-47.**

Canuto, M. M. and Rodero, F. G. (2002). Antifungal drug resistance to azoles and polyenes. *The Lancet Infectious Diseases* **2(9), 550-563.**

Chau, A. S., Mendrick, C. A., Sabatelli, F. J., Loebenberg, D. and McNicholas, P. M. (2004). Application of real-time quantitative PCR to molecular analysis of *Candida albicans* strains exhibiting reduced susceptibility to azoles. *Antimicrobial Agents and Chemotherapy* **48(6), 2124-2131.**

Coleman, D., Sullivan, D. and Moran, G. (2012). *Candida albicans* versus *Candida dubliniensis*: Why Is *C. albicans* more pathogenic. *International Journal of Microbiology* **2012, 205921.**

Dovo, E. E., Zohoncon, T. M., Tovo, S. F., Soubeiga, S. T., Kiendrebeogo, I. T., Yonli, A. T. *et al.* (2022). First detection of mutated *ERG11* gene in vulvovaginal *Candida albicans* isolates at Ouagadougou/Burkina Faso. *BMC Infectious Diseases* **22(1), 678.**

Dufresne, S. F., Marr, K. A., Sydnor, E., Staab, J. F., Karp, J. E., Lu, K. *et al.* (2014). Epidemiology of *Candida kefyr* in patients with hematologic malignancies. *Journal of Clinical Microbiology* **52(6), 1830-1837.**

Feng, W., Yang, J., Xi, Z., Qiao, Z., Lv, Y., Wang, Y. *et al.* (2017). Mutations and/or overexpressions of *ERG4* and *ERG11* genes in clinical azoles-resistant isolates of *Candida albicans*. *Microbial Drug Resistance* **23(5), 563-570.**

Flowers, S. A., Colón, B., Whaley, S. G., Schuler, M. A. and Rogers, P. D. (2015). Contribution of clinically derived mutations in *ERG11* to azole resistance in *Candida albicans*. *Antimicrobial Agents and Chemotherapy* **59(1), 450-460.**

Forche, A., Magee, P. T., Magee, B. B. and May, G. (2004). Genome-wide single-nucleotide polymorphism map for *Candida albicans*. *Eukaryotic Cell* **3(3), 705-714.**

Goldman, G. H., Da Silva Ferreira, M. E., Dos Reis Marques, E., Savoldi, M., Perlin, D., Park, S. *et al.* (2004). Evaluation of fluconazole resistance mechanisms in *Candida albicans* clinical isolates from HIV-infected patients in Brazil. *Diagnostic Microbiology and Infectious Disease* **50(1), 25-32.**

Gubbins, P. O. and Anaissie, E. J. (2009). Antifungal therapy. Clinical Mycology Edn. 2nd. Churchill Livingstone **161-195.**

Jawetz, E., Melnick, J. L. and Adelberg, F. A. (1996). Mikrobiologi Kedokteran. EGC, Jakarta, Indonesia. **pp. 609-610.**

Jain P., Khan Z. K., Bhattacharya E. and Ranade S. A. (2001). Variation in random amplified polymorphic DNA (RAPD) profiles specific to fluconazole-resistant and -sensitive strains of *Candida albicans*. *Diagnostic Microbiology and Infectious Disease* **41(3), 113-119.**

Kordalewska, M. and Perlin, D. S. (2019). Molecular diagnostics in the times of surveillance for *Candida auris*. *Journal of Fungi* **5(3), 77.**

Löffler, J., Kelly, S. L., Hebart, H., Schumacher, U., Lass-Flörl, C. and Einsele, H. (1997). Molecular analysis of cyp51 from fluconazole-resistant *Candida albicans* strains. *FEMS Microbiology Letters* **151(2), 263-268.**

Madani, A. F. and Harahap, M. (2000). Infeksi jamur kulit. Penerbit Hipokrates, Jakarta, Indonesia. **pp. 73-84.**

Mane, A., Vidhate, P., Kusro, C., Waman, V., Saxena, V., Kulkarni-Kale, U. *et al.* (2016). Molecular mechanisms associated with fluconazole resistance in clinical *Candida albicans* isolates from India. *Mycoses* **59(2), 93-100.**

Marichal, P., Koymans, L., Willemsens, S., Bellens, D., Verhasselt, P., Luyten, W. *et al.* (1999). Contribution of mutations in the cytochrome P450 14α-demethylase (Erg11p, Cyp51p) to azole resistance in *Candida albicans*. *Microbiology* **145(10), 2701-2713.**

Marsaoly, R. R., Hari, E. D., Ariwangsa, G. N. A., Karmila, I. D. and Adiguna, M. S. (2014). Profil Dermatomikosis Superfisialis Pada Pasien Geriatri Di Poliklinik Kulit Dan Kelamin Rsup Sanglah Denpasar Bali Periode Tahun 2010-2014(Thesis research in Universitas Udayana). **1, 1-8.**

Mohammadi, Z. and Shalavi, S. (2014). Antifungal effects of root canal irrigants and medicaments. An update review. *The New York State Dental Journal* **80(5), 58-63.**

Morais, P., Adachi, H. and Yu, Y. T. (2020). Suppression of nonsense mutations by new emerging technologies. *International Journal of Molecular Sciences* **21(12), 4394.**

Oliveira, J. M. V., Oliver, J. C., Dias, A. L. T., Padovan, A. C. B., Caixeta, E. S. and Ariosa, M. C. F. (2021). Detection of *ERG11* overexpression in *Candida albicans* isolates from environmental sources and clinical isolates treated with inhibitory and subinhibitory concentrations of fluconazole. *Mycoses* **64(2), 220-227.**

Munin, E., Giroldo L. M., Alves L. P. and Costa M. S. (2007). Study of germ tube formation by *Candida albicans* after photodynamic antimicrobial chemotherapy (PACT). *Journal of Photochemistry and Photobiology B: Biology* **88(1), 16-20.**

Malays. J. Microbiol. Vol 21(4) 2025, pp. 493-501
DOI: http://dx.doi.org/10.21161/mjm.230419

Muzzey, D., Schwartz, K., Weissman, J. S. and Sherlock, G. (2013). Assembly of a phased diploid *Candida albicans* genome facilitates allele-specific measurements and provides a simple model for repeat and indel structure. *Genome Biology* **14(9), 1-14.**

Nurdin, R. S. C., Vitayani, S., Amin, S., Kadir, D., Djamaluddin, W. and Adriani, A. (2021). Cutaneous candidiasis caused by *Candida kefyr*. *The Pan African Medical Journal* **38, 178.**

Nurrachmat, M. and Fiqnasyani, S. F. (2022). Profile of candidiasis patients in rsud Dr Moewardi Surakarta January 2016-December 2019. *Medicinus* **35(2), 10-19.**

Partha, A. D. S. L., Widodo, A. D. W. and Endraswari, P. D. (2022). Evaluation of fluconazole, itraconazole, and voriconazole activity on *Candida albicans*: A case control study. *Annals of Medicine and Surgery* **84, 104882.**

Perea, S., López-Ribot, J. L., Kirkpatrick, W. R., McAtee, R. K., Santillán, R. A., Martínez, M. *et al.* (2001). Prevalence of molecular mechanisms of resistance to azole antifungal agents in *Candida albicans* strains displaying high-level fluconazole resistance isolated from human immunodeficiency virus-infected patients. *Antimicrobial Agents and Chemotherapy* **45(10), 2676-2684.**

Pfaller, M. A. and Diekema, D. (2007). Epidemiology of invasive candidiasis: A persistent public health problem. *Clinical Microbiology Reviews* **20(1), 133-163.**

Pratiwi, S. T. (2008). Mikrobiologi farmasi. *Jakarta: Erlangga* **95, 191.**

Rosana, Y., Yasmon, A. and Lestari, D. C. (2015). Overexpression and mutation as a genetic mechanism of fluconazole resistance in *Candida albicans* isolated from human immunodeficiency virus patients in Indonesia. *Journal of Medical Microbiology* **64(9), 1046-1052.**

Rusu, E., Radu-Popescu, M., Pelinescu, D. and Vassu, T. (2014). Treatment with some anti-inflammatory drugs reduces germ tube formation in *Candida albicans* strains. *Brazilian Journal of Microbiology* **45(4),1379-1383.**

Sanglard, D., Ischer, F., Koymans, L. and Bille, J. (1998). Amino acid substitutions in the cytochrome P-450 lanosterol 14α-demethylase (CYP51A1) from azole-resistant *Candida albicans* clinical isolates contribute to resistance to azole antifungal agents. *Antimicrobial Agents and Chemotherapy* **42(2), 241-253.**

Sofyan, A. and Hikmah Buchair, N. (2022). Penyakit kulit dan kelamin akibat infeksi jamur di poliklinik rsud undata palu tahun 2013-2021. *Journal Kesehatan Masyarakat* **13(2), 384-392.**

Song, J. L., Harry, J. B., Eastman, R. T., Oliver, B. G. and White, T. C. (2004). The *Candida albicans* lanosterol 14-alpha-demethylase (*ERG11*) gene promoter is maximally induced after prolonged growth with antifungal drugs. *Antimicrob Agents Chemother,* **48(4), 1136-1144.**

Strzelczyk, J. K., Ślemp-Migiel, A., Rother, M., Gołąbek, K. and Wiczkowski, A. (2013). Nucleotide substitutions in the *Candida albicans ERG11* gene of azole-susceptible and azole-resistant clinical isolates. *Acta Biochimica Polonica* **60(4), 547-552.**

Wang, H., Kong, F., Sorrell, T. C., Wang, B., McNicholas, P., Pantarat, N. *et al.* (2009). Rapid detection of *ERG11* gene mutations in clinical *Candida albicans* isolates with reduced susceptibility to fluconazole by rolling circle amplification and DNA sequencing. *BMC Microbiology* **9, 1-12.**

Widhiastuti, F., Handamari, D. A. and Musy, R. (2023). Studi retrospektif kunjungan pasien baru mikosis superfisialis di poliklinik kulit dan kelamin RSUD Dr. Soedono Madiun, Indonesia. *Cermin Dunia Kedokteran* **50(4), 186-190.**