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# Quantitative PCR Analysis of FKS1 Gene Expression in Candida glabrata Isolated from Clinical Specimens in Al-Baghdad Governorate

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**ABSTRACT:** The Candida glabrata has become a major opportunistic pathogen, and there is growing resistance to echinocandins, mainly through the changes in FKS1 gene. This paper took on the objective of quantifying the expression of FKS1 gene in C. glabrata isolates of Al-Diwaniyah Province, Iraq, and testing its relationship with antifungal susceptibility. 43 isolates were obtained in the urine and bloodstream and wound samples. They were identified by conventional and automated techniques. The expression of FKS1 was measured using quantitative real-time PCR (qPCR), by normalizing to the expression of the housekeeping gene of ACT1 in the $2^{-\Delta\Delta Ct}$ method. Per CLSI guidelines, caspofungin, micafungin and anidulafungin antifungal susceptibility was also determined. High levels of FKS1 expression (>2-fold) were seen in urinary isolates and were strongly correlated with a decreased sensitivity to echinocandins ($p < 0.05$). Blood isolates recorded the highest resistance rates, in particular, to micafungin and anidulafungin. Prior antifungal exposure was noted to be the most important risk factor related to increments in gene expressions. The results of these studies indicate the involvement of FKS1 upregulation in echinocandin resistance and the importance of molecular surveillance and optimized antifungal stewardship measures to enhance clinical outcomes.

## 1. INTRODUCTION

The current issue of *Candida glabrata* is regarded as one of the most problematic *Candida* species that are not *albicans*. It makes invasive fungus infections all around the globe, and it has turned out to be a huge clinical difficulty to the health care workers [1]. *C. glabrata* also has its share of tricks, including stability of its haploid genome, and this aggravating ability to develop resistance to antifungals, especially the echinocandin family [2]. The bug attacks, particularly, immunocompromised patients, people that have spent more than six weeks in a hospital, and those who have received antifungal treatment previously [3]. *C. glabrata* infection is on the rise in clinical set ups all over the world with high morbidity and mortality rates being witnessed especially in the case of those who are severely ill and those who are immunocompromised. The development of multidrug-resistant isolates, especially to echinocandins, has become a significant clinical challenge with echinocandins commonly being the initial treatment of invasive candidiasis [9,10].

Even with the development of molecular diagnostics, the resistance mechanism, such as that of gene regulation through expression, is poorly understood in most parts of the world. *Candida glabrata* is less sensitive to azole antifungal medications and can develop resistance mechanisms during treatment, making treatment of the fungus more complex and leading to a poor patient prognosis [4]. This tendency has seen an assuring growth over the last 20 years, especially in intensive care units and among patients with hematological malignancies [5]. This increase can be mostly explained by the common use of fluconazole prophylaxis that leads to selective pressure that encourages the growth of less susceptible non-albicans *Candida*

species [6]. *C. glabrata* presents itself in the clinic in all types of infections, such as bloodstream, urinary tract, disseminated candidiasis [7].

The mortality rate is very high and in cases of severely sick patients, it sometimes reaches as high as 40%. Hence there is a dire need to create better diagnostic and treatment interventions that will be useful in controlling these infections [8]. The most frequently used anti-mycosis options against *Candida glabrata* are the echinocandins, namely caspofungin, micafungin and anidulafungin [9]. Their mechanism of action is through inhibiting the production of 1-,3-D-glucan, necessary to the fungal walls. The enzyme complex is the FKS [10]. The FKS1 gene is the gene related to the catalytic subunit of the 2,3-D-glucan synthase complex and mutations in the gene are one of the main mechanisms underlying the resistance to echinocandin [11]. The mutations in certain hotspots repress the binding ability of the enzyme with the echinocandins leading to reduced or total resistance [12]. Gene expression analysis-based improved diagnostics should also be employed, in conjunction with stewardship, infection control, and surveillance to tackle emerging echinocandin resistance [13].

Quantitative PCR has truly transformed the molecular diagnostics scene. It is reproducible, sensitive and specific and therefore it is appropriate in the study of antifungal resistance mechanisms [14,15]. The pattern of transcriptional regulation of FKS1 expression can provide an understanding of the associations between antifungal resistance patterns and transcriptional regulation [16]. Nevertheless, it is not yet clear whether the major cause of the resistance is influenced by the changes in the level of gene expression or the genetic mutations. The answers to these questions are necessary in order to develop effective treatment strategies [17]. FKS1 mutations are known factors leading to echinocandin resistance, but few studies in Iraq have also examined the impact of differential FKS1 gene expression in causing resistance to echinocandins among local isolates. The identification of these patterns may help shed light on the failure of the treatment and inform region-specific antifungal stewardship. The patterns of resistance are very regional. Practices of local prescriptions and infection control vary significantly [18].

In Iraq, especially in Al-Diwaniyah Province, there is a lack of epidemiological and molecular data on *C. glabrata*. Clinicians have no idea on how to treat effectively unless they have the knowledge of the patterns of antifungal resistance in the area and the underlying pathophysiology [19]. The identified gap is a major issue because a lack of this information imposes restrictions on making informed clinical decisions and effectively practicing antifungal stewardship [20]. To address the growing alarm with escalating cases of echinocandin resistance combined with microorganisms resistant to *C. glabrata* treatment having limited therapeutic options, an examination of FKS1 treatment levels in local isolates is of paramount significance [21]. The proposed study will measure FKS1 gene expression in *C. glabrata* isolates of clinical samples in Al-Diwaniyah Province and assess its association with susceptibility to echinocandins. Knowledge on these molecular mechanisms will aid in streamlining of diagnostic guidelines, guide specific antifungal therapy, and enhance local antifungal stewardship activities and eventually enhance patient outcomes.

### *1.1 Research Questions*

RQ1: How does FKS1 gene expression correlate with echinocandin resistance in *Candida glabrata* isolates?

RQ2: How does FKS1 expression vary across different clinical specimen types in *Candida glabrata*?

## 2. LITERATURE REVIEW

### *2.1 Epidemiology and Clinical Significance of Candida glabrata*

The problem of fungal infections has recently become a huge health issue of people all over the world and immunocompromised people, in particular. *Candida albicans Candida glabrata* is one of the most clinically important non-*albicans Candida* species, which has increased in the last few years, and which inherently resists all antifungal agents. According to epidemiological investigations, *C. glabrata* is rapidly emerging as the second-leading cause of candidemia in various regions particularly in developed health care systems [51]. Such a change is mostly associated with the widespread use of azole antifungals which have selective pressure on less susceptible species [5,6]. Clinical burden of *C. glabrata* infection is significant because the infections have been linked with high mortality rate and increased stay in hospitals [7]. The recent findings in surveillance have established that the resistance level of mostly used antifungals continues to raise questions in the treatment strategies [1,9]. In addition, *C. glabrata* has distinct biological traits including haploid genome and speedy adaptability to

adverse factors like antifungals, enhancing its resistance in clinical environments [2,17]. All these are the reasons why there is an urgent necessity to have a more in-depth study of the molecular mechanisms of its pathogenicity and resistance.

*2.2 Mechanisms of Echinocandin Resistance and Role of FKS Genes*

Research has been undertaken over the last ten years that has continued to point out mutations in the FKS genes as the major echinocandin resistance mechanism in *C. glabrata*. The FKS1 and FKS2 genes encode subunits of β-1,3-D-glucan synthase, which is the molecular target of echinocandin drugs. Mutation of certain very hotspots of these genes excludes the drug binding, which causes increases in minimal inhibitory concentrations (MICs) [51]. Classical models focused on point mutations, recent genomic research has shown that mutations that are not in hotspots and other regulatory mechanisms also play a role in resistance [52]. Furthermore, resistance may develop in treatment, which means that it is a dynamic process dependent on the exposure to drugs as well as the host environment. Clinical case studies have demonstrated the possibility of resistance developing within a few weeks following the use of echinocandins, especially in high-risk patients [53]. The results would not uphold the classic definition of resistance as a merely genetic phenomenon and would indicate that the process is multifactorial, with both genetic and adaptive factors. This developing insight highlights the need to incorporate molecular diagnostics with traditional susceptibility testing.

*2.3 Gene Expression and Transcriptional Regulation in Antifungal Resistance*

Previous studies have increasingly focused on how genes are expressed in antifungal resistance, in particular in the elevation of FKS1, and associated pathways. Although mutations still remain in the spotlight, multiple studies have shown that augmentation of resistance-linked genes may be realized even without any mutations being detected. An example illustrating this is that it has been demonstrated by recent experimental studies that FKS1 expression correlates with decreased echinocandin susceptibility, which is an indication of a transcriptional role in resistance. This phenomenon suggests that gene expression modifications, occur as an early form of adaptation, in advance of the formation of stable genetic mutations. This parallels the binary categorization of isolates as either being susceptible or resistant and brings in the intermediate levels of tolerance. Moreover, there is evidence suggesting that regulatory pathways like calcineurin signaling and stress-response networks also play a role in the regulation of the expression of FKS genes, further complicating the matter. Although these achievements have been made, there are big gaps in the literature. Numerous research relies on in vitro models and the clinical evidence correlating the levels of gene expression with patient outcomes is minimal. Thus, transcriptional regulation is becoming a phenomenon with an increasingly recognized importance although its clinical implications are not fully understood.

*2.4 Advances in Molecular Diagnostics: Role of qPCR and Genomic Approaches*

Technological developments have greatly enhanced the process of identifying and describing antifungal resistance as a result of human efforts. Quantitative PCR (qPCR) has become an influential instrument to quantify the amount of gene expression, and it is sensitive, specific, and reproducible [14,15]. Research has shown that assays using qPCR could identify FKS1 mutations and quantitate changes in expression within a few hours; thus, it was possible to detect resistant strains sooner [23,24]. Besides qPCR, whole-genome sequencing (WGS) has also helped gain a better understanding of the genetic landscape of *C. glabrata* [17]. Recent genomic findings have also demonstrated intra-host evolution of the resistance by the weak strains which evolves during a period under antifungal pressure [51, 52]. This shows how resistance is dynamic, and it is important to monitor it over time. The critical review of the literature however reveals that such sophisticated techniques are still not popular in the normal clinical practice, at least in resource constrained contexts. Some of these barriers are high costs, technical complexity, and unstandardized protocols [16]. As a result, there is a gap that requires to be address to fully realize the potential of using the technologies in the clinical care of a patient.

*2.5 Clinical Implications and Future Research Directions*

In all clinical trials, the alarming effects of emerging echinocandin resistance in *C. glabrata* are highlighted. Issues of treatment failure, higher mortality, and restricted therapeutic choices are of high concern. It is worth mentioning that the existing antifungal courses have been defined as a major risk factor in the development of a resistance, and the importance of antifungal stewardship programs is outlined to reduce this problem [54]. Recent practice guidelines might not sufficiently tackle the complexity of resistance mechanisms, especially the contribution of gene expression and reactions [17,38]. Also, local differences in resistance trends point to the need to consider local surveillance data, which is limited in many areas of the

world [48,50]. The future studies should be aimed at incorporating genomic, transcriptomic and clinical data to come up with a more detailed picture of resistance [16,17]. Research to determine the interaction between genetic and regulatory processes (and), in particular, requires studies that uniquely make use of mutation analysis and gene expression profiling [28,39]. Besides, it is possible that new antifungal agents and combination therapy should be developed to deal with the threat of multidrug-resistant *C. glabrata*, which becomes more difficult as a problem [5,33].

## 3. MATERIALS AND METHODS

### *3.1 Study Design and Population*

The study was a cross-sectional one carried out at two large hospitals, Afak Hospital and the Specialized Center for Burns in Diwaniyah, located in Al-Diwaniyah Province between November 2024 and May 2025. The aim of the study was to measure the expression levels of the FKS1 gene in *Candida glabrata* isolates taken out of the clinical samples. Ethics committees of the involved hospitals and the Ministry of Health and the University of Karbala College of Medicine gave ethical approval.

### *3.2 Sample Collection*

Clinical specimens of urine, blood and wound swabs were collected on suspected patients of having fungal infections. Urine samples were gathered in the form of mid-stream samples in well-sterilized bottles whereas blood samples were gathered using venipuncture into blood culture bottles. Sampling of wound was done by using sterile swabs. All the samples were transported to the Microbiology lab at the Afak Hospital as quickly as possible to be processed and analyzed.

### *3.3 Fungal Isolation and Identification*

The specimens were cultured in Sabouraud dextrose agar, CHROMagar *Candida* medium and inoculated with an aerobic culture of 35 o C incubations of 48-72 hours. Identification of the mirrored colonies that seemed to be Candida species was initially based on colony form, color in the chromogenic plates and Gram microscopic stools. The identification to; VITEK-2 automated system was utilized to identify species-level identification with YST identification cards and a subsequent validation of the results by use of traditional germ tube tests and sugar assimilation tests [22].

### *3.4 RNA Extraction and cDNA Synthesis RNA Extraction and cDNA Synthesis.*

The TRIzol reagent method was used to extract total RNA in *Candida glabrata* isolates. Out of the fresh cultures which were growing in the yeast extract peptone dextrose broth, fresh cells were taken and the RNA was extracted according to the manufactures protocol. Gel electrophoresis with agarose gel was employed to authenticate the integrity of the RNA by gel electrophoresis but the concentration was determined using a NanoDrop spectrophotometer. Pollution of genomic DNA was avoided by adding DNase I in the overall RNA preparation and purification. A reverse transcription kit with random hexamers was used to synthesize complementary DNA (1-1μg total RNA) as per the instructions of the user [23].

### *3.5 Quantitative PCR Analysis*

Projected PCR was performed on a real-time PCR thermocycler having SYBR Green-based detection system. FKS1 target primers were selected based on the published sequences and ACT1 was selected as a housekeeping gene to be used as an endogenous control in order to normalize the results. Each tube had a reaction mixture comprising of 10dl SYBR Green MasterMix, 0.5dl forward and reverse primers, 2dl cDNA template and nuclease-free water totalling 20dl. The thermocycling conditions included: 10 minutes of denaturation at 95 C, 40 repetition cycles wherein denaturation of samples were at 95 C and 15 sed at 1 minute, annealing and extension were at 60 C and 1 minute respectively. To ensure the specificity of amplification, melting curve analysis was done. The sample was triply run and the 2- 8Ct system was adopted to demonstrate the comparative expression of the genes [24].

### *3.6 Antifungal Susceptibility Testing*

The broth microdilution technique described in Clinical and Laboratory Standards Institute guidelines [25] was used to determine anti-fungal susceptibility test. Echinocandins tried were caspofungin, micafungin and anidulafungin. Checks of minimum inhibitory concentrations were 24 hours after incubation at 35 C and isolates were defined as susceptible, intermediate or resistant by the set clinical breakpoints [26].

### *3.7 Statistical Analysis*

Statistical analysis was done using SPSS version 26. Demographic and clinical data were summarized by descriptive statistics. As it was determined to be suitable, Mann-Whitney U test or Krustal-Wallis test was used to compare the levels of FKS1 expression between groups. Correlation between the FKS1 expression and minimum inhibitory concentrations was measured using Spearman correlation coefficient. The significant p-value was considered to be below 0.05 [27].

*3.8 Ethical Considerations*

This study received the approval of the Institutional Review Board of the University of Karbala College of Medicine. Informed consent (written and signed) was signed by the participants (or legal guardians), prior to taking samples. The patients remained confidential during the course of the research and all the data were anonymized before analyzing them.

## 4. RESULTS

Table 1 shows the distribution of *Candida glabrata* infections based on age and sex. The most affected patients were between the ages of 33 and 48 years, with 41.9% of the cases, whereas other age brackets accounted 27.9%. The lowest infection rate of 14.0% was observed among the youngest age group (1-16 years), and the highest rate of 16.3% was among the oldest age group (49-64 years). A statistical analysis showed that there was a significant relationship between age and infection rate with a p-value of 0.0012. In terms of sex distribution, males had a greater rate of 60.5% infection than 39.5% in females and this was statistically significant with p-value of 0.0089.

Table 1. *Candida glabrata* infection according to the sexes and ages of patients

| ***Candida glabrata* appearance No. (%)** | | | | | |
|---|---|---|---|---|---|
| **Age group** | | | | **Total** | **P value** |
| **1-16 y** | **17-32 y** | **33-48 y** | **49->64 y** | | |
| **6 (14.0%)** | 12 (27.9%) | 18 (41.9%) | 7 (16.3%) | 43 (100%) | 0.0012* |
| | | | | | |
| **Sex** | | | | **Total** | **P value** |
| **Male** | | Female | | | |
| **26 (60.5%)** | | 17 (39.5%) | | 43 (100%) | 0.0089* |
| ***Significant difference at the 0.05 level by chi-square test** | | | | | |

**Figure 1** shows the relative levels of FKS1 gene expression in various types of clinical specimens. Isolates of urine had the highest median FKS1 expression levels, isolates of blood were second, and wounds had the lowest expression. The statistical significance of the differences in the expression of the levels in different specimen types was found to be significant with p-value 0.0003.

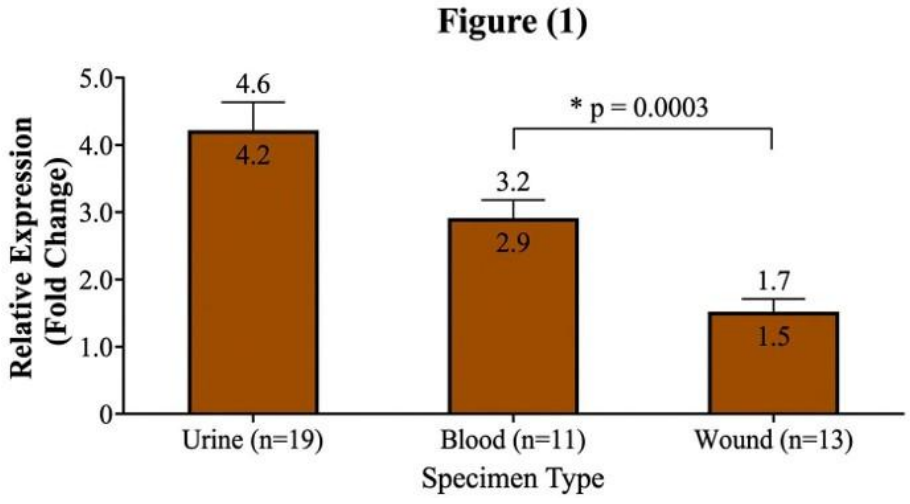


**Figure 1.** Relative FKS1 gene expression in Candida glabrata isolates according to specimen type. Expression levels were normalized to the ACT1 housekeeping gene. Bars represent mean fold change relative to a susceptible reference strain. Significant differences were observed across specimen types (p = 0.0003).

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Table 2 presents the distribution of FKS1 expression status in *Candida glabrata* isolates across different infection types and age categories. Elevated expression (>2-fold) was most frequently observed in UTI isolates from patients aged 17-32 years (46.2%), while normal expression was predominant in wound isolates across all age groups. Statistically significant differences were observed between all types of infections (p = 0.0001).

**Table 2**. FKS1 expression status for *Candida glabrata* in patients according to age category

| Infection types | FKS1 expression status | Age category No. (%) | | | | Total | P value |
|---|---|---|---|---|---|---|---|
| | | 1-16 y | 17-32 y | 33-48 y | 49->64 y | | |
| **UTI (n=19)** | Elevated (>2-fold) | 2 (15.4%) | 6 (46.2%) | 3 (23.1%) | 2 (15.4%) | 13 | 0.0001* |
| | Normal (≤2-fold) | 1 (16.7%) | 2 (33.3%) | 2 (33.3%) | 1 (16.7%) | 6 | 0.0001* |
| **Bloodstream (n=11)** | Elevated (>2-fold) | 1 (16.7%) | 2 (33.3%) | 2 (33.3%) | 1 (16.7%) | 6 | 0.0001* |
| | Normal (≤2-fold) | 1 (20.0%) | 2 (40.0%) | 1 (20.0%) | 1 (20.0%) | 5 | 0.0001* |
| **Wound (n=13)** | Elevated (>2-fold) | 1 (25.0%) | 2 (50.0%) | 1 (25.0%) | 0 (0%) | 4 | 0.0001* |
| | Normal (≤2-fold | 2 (22.2%) | 4 (44.4%) | 2 (22.2%) | 1 (11.1%) | 9 | 0.0001* |

***Significant difference at the 0.05 level by chi-square test**

Table 3 presents the antifungal susceptibility patterns of *Candida glabrata* isolates of various types of infections. Isolates in the bloodstream had the highest rates of resistance to micafungin (45.5%) and anidulafungin (45.5%), whereas the UTI isolates had the lowest resistance rates to all the tested echinocandins (84.2% with caspofungin, 78.9% with micafungin, and 89.5% with anidulafungin). Statistical significance ($p < 0.05$) was used to test all the differences.

**Table 3.** Antifungal susceptibility for *Candida glabrata* in patients according to the type of infection

| Antifungal Agent | R/I/S | Site infection No. (%) | | | Total | P value (p ≤ 0.05) |
|---|---|---|---|---|---|---|
| | | UTI | Bloodstream | Wound | | |
| **CASPOFUNGIN** | R | 3 (15.8%) | 4 (36.4%) | 2 (15.4%) | 9 (20.9%) | 0.0074* |
| | S | 16 (84.2%)* | 7 (63.6%) | 11 (84.6%) | 34 (79.1%) | 0.0001* |
| **MICAFUNGIN** | R | 2 (10.5%) | 5 (45.5%)* | 2 (15.4%) | 9 (20.9%) | 0.0001* |

| | | | | | | |
|---|---|---|---|---|---|---|
| | I | 2 (10.5%) | 1 (9.1%) | 1 (7.7%) | 4 (9.3%) | 0.0001* |
| | S | 15 (78.9%)* | 5 (45.5%) | 10 (76.9%) | 30 (69.8%) | 0.0001* |
| **ANIDULAFUNGIN** | R | 2 (10.5%) | 5 (45.5%)* | 2 (15.4%) | 9 (20.9%) | 0.0001* |
| | S | 17 (89.5%)* | 6 (54.5%) | 11 (84.6%) | 34 (79.1%) | 0.0001* |

***Significant difference at the 0.05 level by chi-square test**

**R: Resistant, I: Intermediate, S: Susceptible**

**Table 4** evaluates the rate of *Candida glabrata* infections in relation to antifungal uptake over different infection types. In UTIs, antifungal uptake was strongly associated with all *C. glabrata* isolates (100%). In bloodstream infections,.636% of patients were previously exposed to antifungals, whereas in wound infections, 61.5% of cases were not previously exposed to antifungals. Statistical significance in the differences was evident in all types of infections.

**Table 4.** Percentage of *Candida glabrata* isolation according to antifungal uptake

| **Type of infection** | ***C. glabrata* infection rate** | | **P value** |
|---|---|---|---|
| ***C. glabrata* infection rate P value** | Non-antifungal uptake cases | Antifungal uptake cases | |
| **UTI (n=19)** | 0 (0%) | 19 (100%) | 0.0001* |
| **Bloodstream (n=11)** | 4 (36.4%) | 7 (63.6%) | 0.0164* |
| **Wound (n=13)** | 8 (61.5%) | 5 (38.5%) | 0.0455* |

***Significant difference at the 0.05 level by chi-square test**

**Table 5** examines the relationship between infection recurrence and prior antifungal use across different sample types. For UTIs, recurrence was strongly linked to antifungal uptake (89.5%), while non-recurrent infections occurred predominantly without prior antifungal use. In bloodstream and wound infections, only non-recurrent cases were observed, with varying proportions of antifungal exposure. All associations were statistically significant.

**Table 5.** Linkage of the recurrence of infection to antifungal uptake in each sample type

| **Site infection** | **Recurrent infection state** | **Antifungal uptake state** | | **P value** |
|---|---|---|---|---|
| | | **Non-antifungal uptake cases** | **Antifungal uptake cases** | |
| **UTI (n=19)** | Non-recurrent | 0 (0%) | 2 (10.5%) | 0.0001* |
| | Recurrent | 17 (89.5%) | 0 (0%) | 0.0001* |
| **P value** | | 0.0001* | 0.0001* | |
| **Bloodstream (n=11)** | Non-recurrent | 4 (36.4%) | 6 (54.5%) | 0.0312* |
| | Recurrent | 0 (0%) | 1 (9.1%) | |
| **P value** | | 0.0001* | 0.0001* | |
| **Wound (n=13)** | Non-recurrent | 8 (61.5%) | 4 (30.8%) | 0.0455* |
| | Recurrent | 0 (0%) | 1 (7.7%) | |

| | | |
|---|---|---|
| **P value** | 0.0001* | 0.0001* |

***Significant difference at the 0.05 level by chi-square test**

**Figure 2** illustrates echinocandin resistance among isolates with elevated FKS1 expression. UTIs had the highest proportion of resistance (71.4%), followed by bloodstream (58.3%), then wounds (42.9%). The differences were statistically significant (p = 0.0001).

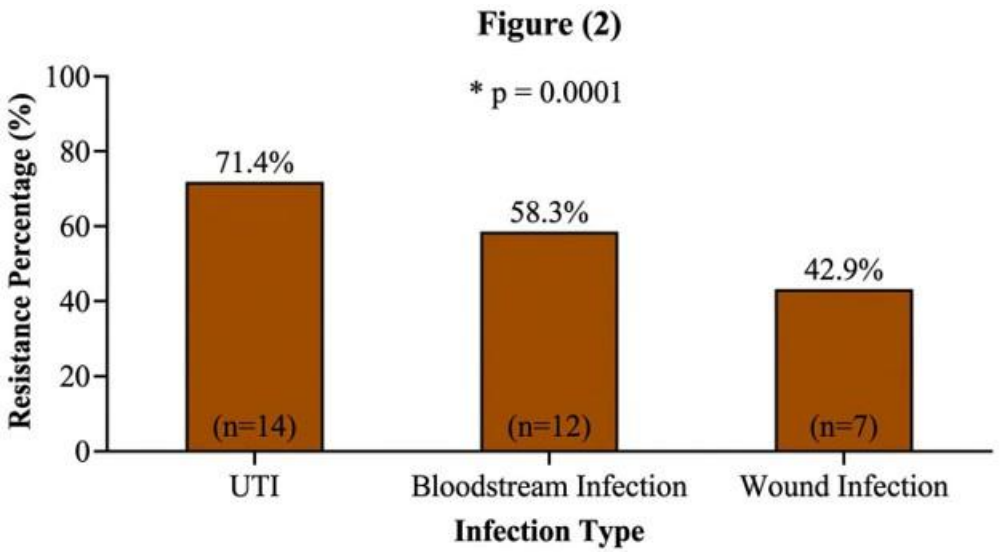


**Figure 1.** Distribution of echinocandin resistance among *Candida glabrata* isolates with elevated FKS1 expression according to infection type. The bars represent the percentage of resistant isolates within each infection category among those showing >2-fold FKS1. Significant differences were observed across infection types (p=0.0001).

## 5. DISCUSSION

FKS1 expression is significantly elevated in a good chunk of *C. glabrata* isolates from Al-Diwaniyah Province. There is a strong link between this upregulation and reduced echinocandin susceptibility [33]. This conclusion will contribute to the accumulating evidence of the role of FKS1 gene expression in antifungal resistance. This poses a great clinical problem considering the fact that few therapeutic measures are available against this pathogen [34]. Looking at demographics, middle-aged adults (33-48 years) had the highest infection rates. That is a bit different from what others have reported. Some studies found more infections in the elderly or in neonates [35]. The observed difference may reflect local hospitalization patterns and the prevalence of underlying conditions such as diabetes, which increase susceptibility to *Candida* infections [36]. Males had more infections than females, 60.5% vs 39.5%. This finding is consistent with previous studies [19] and may be attributed to higher hospitalization rates and invasive procedures among males [37].

The correlation between FKS1 expression and MICs is pretty striking. The correlation coefficients of 0.69 to 0.78 reflect that there is a strong relationship [38]. The growth in the synthesis of the target enzyme might necessitate increased drug levels in order to gain effective inhibition [39]. In line with this, the tendency was found in all three echinocandins, thus suggesting that this upregulation can be one of the reasons behind a wide cross-resistance to this drug category [40]. UTIs showed the highest proportion of isolates with elevated FKS1 expression. Reduced urinary efficacy likely results from poor echinocandin penetration into the urinary tract [41]. Drug levels are often suboptimal there, so the selective pressure for isolates that can tolerate the drug might be stronger. In contrast, bloodstream and wound infections had lower rates of elevated expression, maybe because drug penetration is better in those sites [42]. This clearly points out the fact that the location of infection is significant in the interpretation of the susceptibility results [43].

The presence of prior echinocandin treatment was markedly associated with an increased risk of the high gene expression [44]. This trend is a clear sign of the existence of selective pressure. Weaker correlation with azole was also found but this

might have been an indication of greater antifungal selective pressure or possibly cross-resistance processes which are incompletely understood to date [45,46]. However, this conclusion reinforces the existing principles of antifungal stewardship [47] that requires caution. It is one of the first Iraqi reports looking at the expression of FKS1 in *C. glabrata*. There is a limited amount of regional information that would set a baseline in knowing the resistance mechanisms [48]. The results suggest that there should be enhanced diagnostic instruments and enhanced antifungal stewardship initiative [49,50]. There are some limitations in the study such as a relatively small sample size of 43 isolates. The sample size used would have been more powerful with a larger sample size. FKS1 mutations are another high-ranking antifungal resistance mechanism that was not researched in the study. Prospective research would have integrated the analysis of the expression and mutational screening to have the whole picture.

## 6. CONCLUSION

The FKS1 gene shows increased expression levels in multiple *C. glabrata* isolates from Al-Diwaniyah Province which directly correlates with their decreased ability to withstand echinocandin treatment. The greatest contributors to high expression were UTIs and previous exposure to echinocandins. These results highlight the significance of molecular surveillance of antifungal resistance. Diagnostic methods which involve analysis of the expression of genes as opposed to the classic methods of susceptibility testing are also needed. To cope with the emerging echinocandin resistance, the enhancement of antifungal stewardship, improvement of infection control practices and continuous monitoring of resistance rates should be considered. Future studies need to further investigate the causes of FKS1 upregulation and consider novel treatment options of echinocandin-resistant *C. glabrata* infections.

### *6.1 Recommendations*

According to the results of the current research, it is possible to suggest a number of recommendations aimed at the better management of *Candida glabrata* infections and management of echinocandin resistance. Regular clinical laboratory surveillance of the expression of FKS1 genes has to be a regular activity in clinical labs especially in areas where limited information exists to detect the emergence of resistant isolates and prescribe specific therapy. Antifungal stewardship initiatives should be intensified to reduce the cases of unjust exposure to echinocandin because the use of the drug in the past had a strong correlation with a high level of FKS1 expression and resistance. Furthermore, clinicians are advised to put into consideration specimen type when interpreting susceptibility results, as urinary tract isolates had high levels of expression, implying that there is a problem with drug penetration site specifically. Fourth, these larger scale experiments would need to integrate both gene expression analysis and mutational screening of FKS1 to be able to comprehend more about resistance mechanisms. In addition, ongoing education and training about the correct antifungal prescribing, infection control, and monitoring of resistance that needs to occur to the healthcare personnel to decrease the development and transmission of resistance strains. Inventing and applying quick, affordable diagnostic measures to identify high FKS1 expression on a routine basis will enhance the patient outcomes and maximize treatment approaches.

### *6.3 Limitations*

This study has offered useful insights, but there are a number of limitations that must be recognized. The sample was quite small, and it can constrain the possibility to generalize the results to larger groups. Furthermore, the study concentrated on the expression of FKS1 gene only and failed to examine its mutation, which may give a more in-depth analysis of the mechanism of resistance. In addition, the study used a single geographic area, and differences in clinical practice or antifungal use in other areas could confound findings. However, the limitations also provide clear guidelines on the future research. Increasing sample size and mutational screening and multi-center researches would reinforce evidence and be able to draw more substantive conclusions, which would ultimately have an advantageous effect on clinical management.

### *6.4 Future Implications*

The study of FKS1 gene expression patterns of *Candida glabrata* has important clinical and research implications. These results indicate that regular molecular surveillance should be carried out to identify the earliest evidence of echinocandin resistance, especially in areas with insufficient epidemiological information. Inclusion of gene expression study coupled with the standard testing of vulnerability may enhance treatment of patients by providing focused antifungal treatment. In addition, future research that incorporates the use of expression profiling and mutation screening can help to elucidate the mechanisms behind resistance in a more detailed way. This understanding may guide the creation of new treatment plans and support the

antifungal stewardship programs. FKS1 expression monitoring can help decrease instances of treatment failure and enhance clinical outcome among patients infected with invasive *C. glabrata*.

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**8-DECLARATIONS**

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**9-CREDIT AUTHORSHIP CONTRIBUTION STATEMENT**

Saif T. Jasim : Conceptualization, Investigation, Visualization, Writing – original draft, Writing -

review & editing.

**10-CONFLICTS OF INTEREST**

The authors have no financial or non-financial interests to disclose.

**11-ETHICS STATEMENT**

Written informed consent for publication of clinical details and im-ages was obtained from the patient in accordance with institutional policy. Determined that these -patients cases report did not constitute human subjects research and did not require IRB review

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