

# Prevalence of Vaginal Candidiasis among Pregnant Women Attending Al-Hada Military Hospital, Western Region, Taif, Saudi Arabia

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**Abstract:** This study was intended to determine the prevalence of *Candida albicans* causing vaginitis in pregnant women and their susceptibility to current antifungal drugs from Western Region, Taif, Saudi Arabi. A total 1207 samples of high vaginal swab were collected from pregnant women between the ages of (15-44) years attending ante-natal clinic and between 35 and 37 of weeks of gestation, from February to October 2014. Standard microbiological methods were used to isolate and identify *Candida* from vaginal swabs obtained from study subjects. An antimicrobial susceptibility test was performed for all *Candida* isolates according to the criteria of the Clinical and laboratory Standards Institute. The result was, A total of 848 (70, 2%) isolates were obtained which comprises six different *Candida* species, namely *Candida albicans*, *C. glabrata*, *C. tropicalis*, *Candida lipolytica*, *Candidalutitaniae*, and *Candida kefyr* with frequency of occurrence of (848, 70, 2%), (200, 16.5%), (40, 3.3%), (32, 2.6%), (16, 1.3%), and (8, 0.0.6%) respectively. Different type of non *Candida* were isolated and found in very low percentage *Cryptococcus laurentii*, *Saccharomyces cerevisiae*, *Stephanoascus ferrii*, and *Trichosporon asahii*. All isolates were tested against antifungal agents, and all *Candida* spp., non-*albicans* were susceptible to ketoconazole and Fluconazole. These results were the first record of the database of VVC in pregnant women in Saudi Arabia population at western region, Taif.

**Keywords:** antenatal, *Candida albicans*, *Candida non albicans*, ketoconazole, fluconazol, pregnancy, Vulvovaginal Candidiasis.

## 1. Introduction

Vaginal candidiasis is a fungal infection caused by overgrowth of *Candida* species affecting the genital tract as opportunistic pathogen. Vaginal candidiasis (VC) is a common type of vaginitis, a gynaecologic disorder with a white discharge, soreness, dyspareunia, irritation and itching.[1].Vaginal candidiasis is a common complaint among women of different age groups, regardless of their sexual activities and can be possible risk for other diseases e.g. HIV/AIDS [2-5].This infection progresses as colonization, superficial infection and hematogenous dissemination to different organ [3, 6-7].Reports show that about 75% of all women, experience at least one episode of which physician approved to be candidiasis in their lifetime [5, 8, 9].

*Candida* spp. are the most common cause of fungal infections, leading to a range of life-threatening invasive to non-life-threatening mucocutaneous diseases. Vaginal candidiasis is a frequent companion of pregnancy, which greatly complicates the course of the pregnancy and threatens the health of both mother and child [10].Vaginal candidiasis (VC) is a common type of vaginitis, a gynaecologic disorder with a white discharge, soreness, dyspareunia, irritation and itching. The incidence of vaginal candidiasis is almost doubled (particularly in the second and third trimester) among pregnant women, due to high production or changes in the levels of sex hormones and deposition of glycogen in the vagina during pregnancy [11].Among *Candida* spp., *Candida albicans* the most common infectious agent. Non-*C.albicans* species are emerging pathogens and can also colonize human mucocutaneous surfaces [12]. Consequently, they are also

isolated in the setting of candidiasis, albeit at a lower frequency.

The pathogenesis and prognosis of candidial infections are affected by the host immune status and also differ greatly according to disease presentations. Therefore, diagnosis, management, and treatment choices vary and need to be considered in the overall setting of the affected human host. At least 75% women suffer once in their life from one episode of a candida infection [13-15].Although *Candida albicans* the pathogen identified in most patients with vulvovaginal candidiasis, other possible pathogens include *Candida tropicalis*, *Candida glabrata* amongst others, which are responsible for up to 33 percent of recurrent infections [16-18].

*Candida tropicalis* and *glabrata* are the most important of the non-*C albicans* infections [19, 20].Although some non-*albicans* species like *Candida tropicalis*, *Candidalipolytica*, *Candida lusitaniae*, *Candidakefyr* [21].Also *Cryptococcus laurentii*, *Trichosporon asahii*, *Stephanoascus ferrii*, *Saccharomyces cerevisiae* are recovered from infected individuals, and can cause yeast vaginitis[10, 22].Relatively higher antifungal resistance rate of non- *C albicans* species may contribute to higher rates of recurrent infections. This increase in non- *C. albicans* *Candida* vulvovaginitis has been attributed to overuse of antifungal therapy, which has resulted in the elimination of the more sensitive *C.albicans* and selection of other, more resistant species [23, 24]. Secretory acid proteinase production has been associated with yeast pathogenicity [25]. These enzymes are implicated in the persistence and colonization of the vaginal tract [26-28].They also facilitate tissue penetration and cleavage of immunoglobulin A (IgA), which is an important factor in

vaginal immunity [29]. The lack of specificity of symptoms and signs therefore precludes a diagnosis that is based on history and physical examination without the corroborative evidence of laboratory tests (CDC, 2010) [30].

Thus, over 80% patients, referred by physicians with a putative diagnosis of VVC were found to have some other cause of vaginitis, and therefore most patients fail to respond to antifungal therapy cause of incorrect diagnosis [31]. Therefore, rapid and specific detection and identification of *Candida* species will help to choose the suitable antifungal and improve patient care.

This immune imbalance is caused by a number of factors, such as excess stress, allergies, indiscriminate use of antibiotics, steroids, birth control pills and hormonal drugs and nutrient deficiency [10, 32]. Diabetes mellitus, pregnancy, and the use of tight nylon underwear also enhance overgrowth of *Candida* in a manner that cannot easily be controlled by the body's defense mechanisms [10, 33-37]. The dynamic environment inside the host may include factors such as temperature, pH, and the normal flora and its metabolic products. These factors, along with the oxidative stresses associated with host defense mechanisms, require physiologic adaptation in the pathogenesis of *C. albicans*. Temperature enhances the virulence of several bacteria and influences fungal morphogenesis [38]. Increased temperature, pH, and steroids have also been shown to induce the yeast-to-hyphal transformation of *C. albicans*, 4-6 which in turn is linked to the virulence of this organism [39]. This immune imbalance is caused by a number of factors, such as excess stress, allergies, indiscriminate use of antibiotics, steroids, birth control pills and hormonal drugs and nutrient deficiency [10, 32]. Diabetes mellitus, pregnancy, and the use of tight nylon underwear also enhance overgrowth of *Candida* in a manner that cannot easily be controlled by the body's defence mechanisms [9, 34-36].

This study was intended to determine the prevalence of *Candida albicans* causing vaginitis in pregnant women and their susceptibility to current antifungal drugs from Western Region, Taif, Saudi Arabia. In the Kingdom of Saudi Arabia, there is few of information regarding *Candida albicans* causing vaginitis in pregnant women, in Riyadh [40, 41], Makkah [42], and Al-Madinah Al-Munawarah [43, 44]. This paper was the first data on incidence of *Candida albicans* causing vaginitis in pregnant women at Western Region, Taif, Saudi Arabia.

## 2. Materials and Methods

### 2.1. Study population

A total of (1207) pregnant women attending the routine antenatal clinic of the Al-Hada Armed Forces Hospital were screened for yeast in routine examination. The samples were collected during (9) months, starting from February (2014) to October (2014). The approach was based on universal screening of all pregnant women for yeast infection between 35 and 37 of weeks of gestation [11, 14]. The age of the women's between 15-44 Year

### 2.2. Sample Collection

A total 1207 samples of high vaginal swab were collected from asymptomatic and symptomatic pregnant women between the ages of (15-44) years attending ante-natal clinic. All women who gave a history of taking anti-fungal agents during the earlier 2 weeks prior to the study were excluded from the study. A verbal consent was obtained from each participant who also completed a questionnaire with demographic data and possible risk factors such as age, pregnancy, occupational, diabetes, and history of vaginitis, abortion, antifungal use and smoking [45-49]. Collection of vaginal swab was done by exposing the posterior fornix with a sterile vaginal speculum (Coscos). All genital swabs collected in Amies transport medium received from women during the study period were included. Two vaginal swabs were collected from each patient and transported to the microbiology laboratory at Al-Hada Military Hospital for processing.

### 2.3. Macroscopic examination of samples

Each sample was examined for colour, appearance and odour and described as whitish or whitish-gray colour, cottage cheese-like discharge and odourless. The one swab was used for microscopy for wet preparation and gram stain while the other one was used for culture onto Sabouraud's dextrose agar (Saudi Prepared Media Laboratory, Saudi Arabia, Riyadh (SPML) and incubated at 37° C for 18-24 hours to see the growth of creamy, greyish moist colonies.

### 2.4 Microscopic examination of samples

Saline wet mount preparation of the colony was made to see budding cells and pseudohyphae. The plated were read after incubation and all colonies suspected of having *Candida* morphology were gram stained.

### 2.5. Species identification

The species identification was based on germ tube test, sugar assimilation test, sugar fermentation test according to standard methods described earlier [50], and According to guidelines of the CDC, Sexually Transmitted Diseases Treatment Guidelines (2010) [50]. Identified isolates were stored on nutrient agar slant at room temperature for subsequent susceptibility testing.

### 2.6. Germ-tube test

Small inoculum of suspected *Candida* cultures were inoculated into 1 ml of human serum (Sigma-Aldrich, Germany) in a small tube and incubated at 37° C for 2 hours. After incubation, a loop-full of culture was placed on a glass slide, overlaid with a cover-slip and examined microscopically for the presence or absence of germ-tubes. Formation of germ tubes was seen as long tube like projections extending from the yeast cells with no constriction or septa at the point of attachment to the yeast cells [46-49]. Biochemical identifications were also performed on all isolates, and colonies were identified to species level using Vitek YBC cards (Vitek Systems, BioMerieux).

### 2.7 Susceptibility testing

The isolated yeasts (1207 strains) were also tested for their *in vitro* susceptibility towards fluconazole and

ketoconazole in accordance with the proposed guidelines for antifungal disk diffusion susceptibility testing of yeasts contained in the CLSI document M-44A (CLSI, 2004). A reference strain of *C. albicans* ATCC 25285 was included as control. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the antibiotic that yielded no bacterial growth [51]. The plates were incubated at 35°C, and inhibition zone diameters were measured after 24 and 48 h especially for *C. glabrata*. The interpretive criteria for the fluconazole disk test were as those previously described by CLSI (2004):  $\text{dz} \geq 19\text{mm}$ -susceptible;  $15 < \text{dz} < 18\text{ mm}$ -susceptible dose dependent and  $\text{dz} \leq 14\text{mm}$ -resistant. As for ketoconazole:  $\text{dz} \geq 20\text{mm}$ -susceptible,  $20 < \text{dz} < 10\text{mm}$ -intermediae and  $\text{dz} < 10\text{mm}$ -resistant [52].

## 2.8. Data analysis

Statistical analyses were performed using the Statistical Package for the Social Science (SPSS), Version 16 for Windows. Continuous variables were summarized using descriptive statistics in terms of means  $\pm$  standard deviations; 95% confidence intervals (95% CI), minimums and maximums, while a Chi-square test were used to compare categorical variables. A  $p$ -value  $< 0.05$  were considered significant. The analysis was performed with Statistical Package for Social Sciences (SPSS) software version 15 (SPSS, Inc., Chicago, IL, USA). Prevalence figures were calculated for the total study population and separately by age groups. Occupation, number of antenatal clinic visit (ANC), and type of gravida also recorded. Chisquare test was used to compare results between the pregnant women with different age groups. Statistical methods, descriptive statistics included statements of frequency with percentages, means, standard deviations (SD, and differences were considered significant if  $p < 0.05$  with confidence intervals (95% CI)

## 3. Results

### 3.1. Incidence of vaginal candidiasis among pregnant women

Total of (1207) samples were collected for isolation and identification of *Candida* species and other yeast from both symptomatic and asymptomatic pregnant women. 848 of the 1207 patients examined were positive for *Candida albicans*, which gave an incident rate of 70.2 % (Table 1).

*Candida glabrata* followed in prevalence with carriage rate (200, 16.5%). Other *Candida* species were found in low percentage as follows: *Candida tropicalis* (40, 3.3%), *Candida lipolytica* (32, 2.6%), *Candida lusitanae* (16, 1.3%), *Candida kefyr* (8, 0.6%). Different type of non *Candida* isolated from pregnant women also found in very low percentage as follows *Cryptococcus laurentii*, *Saccharomyces cerevisiae* (24, 1.9%), *Stephanoascus ferrii*, and *Trichosporon asahii* (8, 0.6%).

**Table 1:** Different type of *Candida* species, and non *Candida* isolated from pregnant women ( $n=1207$ )

Different type of <i>Candida</i> isolated from pregnant women		
Name	Frequency	Percent (%)
<i>Candida albicans</i>	848	70.2
<i>Candida glabrata</i>	200	16.5
<i>Candida tropicalis</i>	40	3.3
<i>Candida lipolytica</i>	32	2.6
<i>Candida lusitanae</i>	16	1.3
<i>Candida kefyr</i>	8	0.6
Different type of non <i>Candida</i> isolated from pregnant women		
Name	Frequency	Percent (%)
<i>Saccharomyces cerevisiae</i>	24	1.9
<i>Cryptococcus laurentii</i>	24	1.9
<i>Stephanoascus ferrii</i>	8	0.6
<i>Trichosporon asahii</i>	8	0.6
All Total	1207	100

### 3.2. Socio-demographic characteristics

The socio-demographic characteristics of (1207) pregnant women screened for yeast infection are presented in Table 2.

#### 3.2.1. Age groups in years

The highest occurrence of *Candida* species (68.3%) was recorded between ages 25-29 followed by ages 30-34 with percentage occurrence of (9.6%) while the lowest occurrence (2.4%) was recorded between age group 40-44 (table. 2).

**Table 2:** Socio-demographic characteristics of 1207 women attending obstetric clinic investigated for yeast, at AL-Hada Armed Forces Hospital in Taif, SA, 2014

Socio-demographic Characteristics	Frequency	Percent (%)	P value
<b>1- Age groups in years</b>			
15-19	62	5.1	
20-24	80	6.6	
25-29	825	68.3	
30-34	116	9.6	
35-39	95	7.8	
40-44	29	2.4	
Total	1207	99.8	
<b>2-Occupation</b>			
House wife	94	7.7	
Teacher	925	76.6	
Student	91	7.5	0.04
Nurse	57	4.7	0.01
Doctor	41	3.3	
Total	1207	100	

#### 3.2.2. Occupation

The prevalence of *Candida* was studied in relation to Occupation. The highest occurrence of *Candida* was found in the teacher (76.6%) followed by house wife (94, 7.7%), and student (91, 7.5%)  $P 0.04$ , while the least occurrence was found in the nurse (57, 4.7)  $P 0.01$ , and doctor (41, 3.3%) as shown in Table 2.

The present data shown, there is no statistically significant association of *Candida albicans* was observed in connection with any of the socio- demographic



characteristics of the study subjects by age groups in years. As shown in Table 2

**Table 3:** Variables associated/not associated with *Candida albicans* in pregnant women attending obstetric clinic investigated for yeast, at AL-Hada Armed Forces Hospital in Taif, SA, 2014

Variables	Total N=848	<i>Candida albicans</i>	Percentages (%)	P value
<b>1- Age (in years)</b>				
15-19	61	59	10.1	
20-24	80	71	12.2	
25-29	469	220	37.9	
30-34	115	112	19.3	
35-39	94	91	15.7	0.01
40-44	29	26	4.4	
Total	848	579	100	
<b>2-Occupation</b>				
House wife	151	127	21.9	
Teacher	301	112	19.3	
Student	181	160	27.6	
Nurses	163	151	26	0.007
Doctors	52	29	5	
Total	848	579	100	
<b>3-Number of ANC Visit</b>				
One time	120	118	20.3	
Two times	398	205	35.3	
Three times	203	146	25.2	
Four times	127	110	18.9	0.006
Total	848	579	100	
<b>4-Type of Gravida</b>				
Primigravida	265	198	34.1	
Multigravida	583	381	65.8	
Total	848	579	100	

The highest occurrence of *Candida* species (37.9%) was recorded between ages 25-29 followed by ages 30-34 with percentage occurrence of (19.3%) while the lowest occurrence (4.4%) was recorded between age group 40-44 (table.3).

### 3.2.3. Variables associated/not associated with *Candida albicans* in pregnant women

Most of women under investigation were in age 25-29 (220, 37.9%). The prevalence of *Candida* was studied in relation to Occupation. The highest occurrence of *Candida* was found in the teacher (76.6%) followed by house wife (94, 7.7%), and student (91, 7.5%)  $P=0.04$ , while the least occurrence was found in the nurse (57, 4.7%)  $P=0.01$ , and doctor (41, 3.3%) as shown in Table 2. There is statistically significant association of *Candida albicans* was observed in connection with any of the socio-demographic characteristics of the study subjects by age groups in years 35-39 (91, 15.7%)  $P=0.01$ . There is statistically significant association of *Candida albicans* was observed in connection with any of the socio-demographic characteristics of the study subjects by occupation in nurses (151, 26%)  $P=0.007$

In contrast, there is also statistically significant association of *Candida albicans* was observed in number of ANC Visit in four times (110, 18.9%)  $P=0.006$ . Statistically no significant association of *Candida albicans* was observed in connection with any of the socio-demographic characteristics of the study subjects by type of gravida, either primigravida nor multigravida.

**Table 4:** Comparative susceptibilities of *Candida albicans* and non-*albicans* spp. against fluconazole and ketoconazole

Antifungal-agent	Classified as	<i>C. albicans</i> n=848 (%)	Non- <i>albicans</i> n=359 (%)
Ketoconazole	S	846 (99)	359 (100)
	I	2.0 (0.23)	0.0 (0.0)
	R	0.0 (0.0)	0.0 (0.0)
Fluconazole	S	839 (98)	348 (96.9)
	I	8.0 (0.94)	3.0 (0.83)
	R	1.0 (0.11)	8 (2.22)

Results of this study on the antifungal activity of tested agents (Table 4) indicated that all *Candida* spp. tested against ketoconazole and Fluconazole were susceptible (99%), (98%) of *C. albicans* (1207/848) strains respectively. Non-*albicans* were also susceptible to Ketoconazole and fluconazole (100%), (96.9%) respectively. Exhibited resistance to fluconazole predominantly *C. glabrata*

## 4. Discussion

This study investigated the prevalence of vaginal candidiasis among pregnant women attending antenatal clinic over the period of nine (9) months in Al-Hada Military Hospital, Western Region, Taif, Saudi Arabia. The study showed high prevalence (848, 70.2%) of vaginal candidiasis among pregnant women. The high prevalence of candidiasis reported was similar to the findings of [53, 54, 55] who reported a prevalence rate of 60%, 62.2% and 67% respectively among pregnant women in Jos and Enugu State, Nigeria. The finding is however in contrast to the observations of [33] who reported a frequency of 38%.

The high prevalence of vaginal candidiasis among pregnant women attending antenatal clinic in Al-Hada Military Hospital, may be due to inadequate knowledge, poor personal hygiene, limited diagnostic facilities, poor dietary habits, shortage of effective treatment, increased levels of estrogens and corticoids, wearing of tight-fitting synthetic underclothing, prolonged use of antibiotics which kill the good and beneficial bacteria [33, 56, 57]. This high prevalence may result into the risk of miscarriage, premature birth, fetal oxygen deficiency, low birth weight and neonate mortality, wound infection after childbirth and systemic inflammation. Other possible risks include pelvic inflammatory disease, infertility, pelvic abscess, stress, discomfort and irritation [58]. In the present study the overall prevalence of *Candida albicans* among pregnant women was found to be 70.2%. Similar findings have been reported in Makkah City, Saudi Arabia *C. albicans* affecting 163 (72.4%) [42]. While in Al-Madinah Al-Munawarah incidence of *C. albicans* and carriage rate higher than other cities 98 (81.7%) [43].

The highest prevalence of vaginal infections in this study was noted among the age groups 25-29 years 220 (37.9%), followed by 35-39 age group 91 (15.7%). This report agreed with [59], which reported a peak vaginal infections between age group 20 and 40. This may be due to high sexual activity, poor personal hygiene, the use of contraceptives and drug abuse among this age group. Among the 70.2% pregnant women found to be positive with vaginal candidiasis, 76% were found to be teacher (Occupation), while (91, 7.5%) was found to be Student. In contrast nurses have significant in VVS (57, 4.7%)  $P < 0.01$ . This high prevalence among teacher may be due to the use of contraceptive and antibiotics or other reasons [60].

The data in the present study showed that, Number of ANC Visit (four time) have significant in VVS (110, 18.9%)  $P < 0.006$ . Among the 70.2% pregnant women found to be positive with vaginal candidiasis, 34.1% were found to be primigravidae (women with first pregnancy) while 65.85% was found to be multigravidae (women that has experience of more than one pregnancy). This high prevalence among the multigravidae may be due to the use of contraceptive and antibiotics.

This is similar to the findings of [58] who reported an occurrence of 60% among the multigravidae and 40% among primigravidae among pregnant women (patients) in Department of Pathology, Allama Iqbal Medical College, Lahore- Pakistan. *C. albicans* had the highest occurrence (70.2%), *C. glabrata* found next after *C. albicans* with 16.5% (9), *C. tropicalis* with 3.3% (6), Other *Candida* species were found in low percentage. *Candida tropicalis* (40, 3.3%), *Candida lipolytica* (32, 2.6%), *Candida lusitanae* (16, 1.3%), *Candida kefyr* (8, 0.6%) in this study which is quite comparable with the reports of [61-62]. This is similar to the reports of [63] in India and [64] in Iran.

The high occurrence rate (72.2%) of *C. albicans* observed in this study as shown in figure 2 is an indication that it is a leading causative agent of the reproductive tract yeast infections in women of child bearing age as also observed by [65]. This may be due to its virulent factors which include dimorphism and phenotypic switching. *Candida albicans* produces protease and phosphatase which enhance its attachment to human epithelium.

It can also be deduced that the high incidence rate of *C. albicans* could be due to increased physiological changes, estrogen and rich glycogen content of the vaginal mucosa thereby providing an adequate supply of utilizable sugar that favor its growth during pregnancy. However, Wise *et al.* [66] and Trofaet *et al.* [67] reported a low occurrence of *C. albicans* in New York. The low occurrence of *C. albicans* reported by Wise *et al.* [66] and Trofaet *et al.* [67] may be as a result of good personal hygiene, appropriate nutrition, adequate diagnostic facilities and treatment. Furthermore, our data on the antifungal activity of fluconazole against *C. albicans*, revealed that 98% of tested strains were susceptible. This sensitivity rate is more or less comparable with those reported by [68-70].

In agreement with the study of [71], most of the detected resistant strains belong to *C. glabrata*, emphasizing, and its greatest potential to acquire resistance to fluconazole. Resistance to fluconazole in clinical isolates of *C. tropicalis* has increased [72-74]. The precise mechanisms responsible for drug resistance in *Candida* species have been described [75]. *Saccharomyces cerevisiae*, the baker's yeast, causes disseminated infections in immunocompromised hosts [76, 77]. VVC is infrequently caused by *C. tropicalis*, and *Candida lipolytica*, *Candida lusitanae*, *Candida kefyr*, although most species of *Candida* have been associated with condition [78, 5]. VVC induced by NCAC is clinically indistinguishable from the caused by *C. albicans*; moreover, such species are often more resistant to treatment [79].

## 5. Conclusion

The results of this study showed a high occurrence of *Candida* among pregnant women attending Al-Hada Military Hospital, Western Region, Taif, Saudi Arabia. *C. albicans* was the most prevalent among the isolates with high prevalence of vaginal candidiasis. The overall results of this study emphasize the importance of the different risk factors that play a role in *Candida*-colonization. The culture of vaginal discharge should be warranted because culture technique is more sensitive than direct smear. These findings may help in drawing strategies in preventing and controlling vulvovaginal candidiasis that may affect women and their newborns. Use of fluconazole should not be continued in patients with recurrent *Candida* infections.

## 6. Recommendations

1. Rapid species identification from clinical specimens and standard drug susceptibility testing would be an effective approach for controlling nosocomial outbreaks caused by *C. tropicalis* or CNA species in different clinical settings.
2. Early diagnosis and adequate treatment of vaginal candidiasis should be practiced.
3. Consistent application of standard techniques, guidelines for the use of antifungal agents and control measures for predisposing factors or risk factors may potentially reduce the risk of drug-resistant life-threatening infections.
4. The need for regular check-up at different gestation period should be encouraged.
5. Drug abuse among pregnant women should be discouraged.
6. Application of standardized technology and its consistent use in diagnostics and research still remains a major global challenge.

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